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Abstract

The fumonisins and AAL-toxin are structurally-related natural products produced by fungi (22, 23, 24). Fumonisin B1 [FB1] has been recognized since 1988 as a mammalian toxin (36, 37, 44, 52). More recently, FB1 has been shown to be a phytotoxin as well (1,2,3). AAL-toxin has been known to produce symptoms of stem canker disease on susceptible (*asc*/*asc*) tomatoes (9, 48, 56, 58). Our research has focused on evaluating the potential of the class of natural products defined by AAL-toxin and the fumonisims for the development of improved weed control agents. This work has involved the development of improved methods for the isolation, purification and characterization of these toxins. Useful methods have been developed for assessing phytotoxicity of these toxins with various weed species, and for estimating mammalian toxicity. A series of analogs has been obtained, either derivatizing the parent toxins or by *de novo* synthesis, and evaluated for phytotoxicity and mammalian cytotoxicity. Characterization of the toxins has included studies on their mode of action in plant tissue, their ability to act as sphingosine analogs and their stereochemistry.

Keywords

Absolute Configuration, Mammalian Cell Culture, Alternaria Alternata, Fusarium Moniliforme, Sphingolipid Metabolism

Disciplines

Environmental Chemistry | Organic Chemistry

Comments

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THE CHEMISTRY AND BIOLOGICAL ACTIVITIES OF THE NATURAL PRODUCTS AAL-TOXIN AND THE FUMONISINS

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INTRODUCTION

The fumonisins and AAL-toxin are structurally-related natural products produced by fungi (22, 23, 24). Fumonisin B_1 [FB_1] has been recognized since 1988 as a mammalian toxin (36, 37, 44, 52). More recently, FB_1 has been shown to be a phytotoxin as well (1, 2, 3). AAL-toxin has been known to produce symptoms of stem canker disease on susceptible (*asc/asc*) tomatoes (9, 48, 56, 58). Our research has focused on evaluating the potential of the class of natural products defined by AAL-toxin and the fumonisims for the development of improved weed control agents. This work has involved the development of improved methods for the isolation, purification and characterization of these toxins. Useful methods have been developed for assessing phytotoxicity of these toxins with various weed species, and for estimating mammalian toxicity. A series of analogs has been obtained, either derivatizing the parent toxins or by *de novo* synthesis, and evaluated for phytotoxicity and mammalian cytotoxicity. Characterization of the toxins has included studies on their mode of action in plant tissue, their ability to act as sphingosine analogs and their stereochemistry.

1. Isolation, Purification and Characterization

The genus *Alternaria* is widely distributed in nature, and a number of *Alternaria* spp are pathogenic to plants (66). *A. alternata* f. sp. *lycopersici* was isolated from susceptible (*asc/asc*) tomatoes with stem canker disease (33, 40, 48). The active phytotoxin responsible

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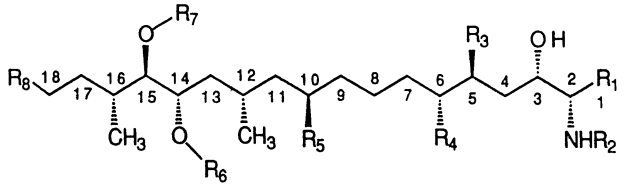


Toxin[a]	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
AAL-TA_1	H	H	OH	OH	H	PTCA	H	H
AAL-TA_2	H	H	OH	OH	H	H	PTCA	H
AAL-TB_1	H	H	OH	H	H	PTCA	H	H
AAL-TB_2	H	H	OH	H	H	H	PTCA	H
AAL-TC_1	H	H	H	H	H	PTCA	H	H
AAL-TC_2	H	H	H	H	H	H	PTCA	H
AAL-TD_1	H	$COCH_3$	OH	H	H	PTCA	H	H
AAL-TD_2	H	$COCH_3$	OH	H	H	H	PTCA	H
AAL-TE_1	H	$COCH_3$	H	H	H	PTCA	H	H
AAL-TE_2	H	$COCH_3$	H	H	H	H	PTCA	C_2H_5
FA_1	CH_3	$COCH_3$	OH	H	OH	PTCA	PTCA	C_2H_5
FA_2	CH_3	$COCH_3$	OH	H	H	PTCA	PTCA	C_2H_5
FB_1	CH_3	H	OH	H	OH	PTCA	PTCA	C_2H_5
FB_2	CH_3	H	OH	H	H	PTCA	PTCA	C_2H_5
FB_3	CH_3	H	H	H	OH	PTCA	PTCA	C_2H_5
FB_4	CH_3	H	H	H	H	PTCA	PTCA	C_2H_5
FC_1	H	H	OH	H	OH	PTCA	PTCA	C_2H_5
HFB_1 (AP_1)	CH_3	H	OH	H	OH	H	H	C_2H_5
HFB_2 (AP_2)	CH_3	H	OH	H	H	H	H	C_2H_5

[a] The indicated absolute configurations have been established only for AAL-TA_1, AAL-TA_2, FB_1, HFB_1 and HFB_2.

Figure 1. Chemical structures of various analogs of AAL-toxins isolated from *A. alternata* f. sp. *lycopersici*, various analogs of fumonisins A and B isolated from *F. moniliforme*, the hydrolyzed products (AP_1 and AP_2) and the absolute configurations of AAL-TA_1, AAL-TA_2, FB_1, HFB_1 and HFB_2.

for stem canker was found to be AAL-toxin (9,22, 23, 33). AAL-toxin was originally isolated from liquid fermentation cultures of *A. alternata* f. sp. *lycopersici* by thin-layer chromatography (22, 23). Two compounds were isolated, TA and TB, each having two structural isomers (Fig. 1). Since then TC, TD and TE, each with two isomers, have been discovered (28).

The AAL-toxins are hydroxylated, long-chain alkylamines with one esterified propane-1,2,3-tricarboxylic acid moiety. The difference between TA and TB is that TB lacks a hydroxyl group at R_4 (Fig. 1). TA and TB are equally active against susceptible tomatoes (56, 58). However, removal of the hydroxyl group at R_3, which produces TC, renders the compound much less active (300 ng vs. 10 ng per plant to cause visible symptoms) (28). TD and TE are acetylated forms of TB and TC, respectively. They represent up to 40% of the AAL-toxins produced by fermentation in liquid medium. TD and TE are essentially inactive in phytotoxicity testing and are probably metabolic products from detoxification of the active metabolites (28).

We have developed a fermentation process for *A. alternata* and other fungi on solid media, particularly rice (5, 8). The procedure is briefly described as follows. Rice was moistened with water and shaken and autoclaved twice. The flasks were inoculated with *A. alternata* NRRL 18822 grown on potato dextrose agar. Flasks were incubated for 4 wks in 12 h light/12 h dark at 28 C.

AAL-toxin was extracted from rice by initially pre-extracting with chloroform overnight and discarding the lipid extract. The lipid-free residue contained the AAL-toxin which was extracted three times with a water:methanol (60:40 v/v) mixture. Evaporation of the methanol leaves AAL-toxin in water, which is applied to an Amberlite XAD-2 column. Elution with methanol yields crude AAL-toxin which can be purified by flash column chromatography on reverse-phase C_{18} packing eluted with 60% aqueous methanol. This produces AAL-toxin isomer TA as a white solid at ≥ 95% purity determined by TLC. The advantage of this method is isolation of a pure compound, which can be used for standardized phytotoxicity testing. All of our subsequent studies have been done using purified AAL-toxin isomer TA.

The fumonisins are related structurally to AAL-toxin (Fig. 1), but are produced by *Fusarium moniliforme*, *F. proliferatum* and other *Fusaria* of the Liseola section (57, 76). Recently, it has been reported by Chen et al., (31) that small amounts of FB_1 were produced by *Alternaria alternata* f. sp. *lycopersici* as well, although this finding has been disputed by others. Recently, Abbas and Riley (14) reported that *A. alternata* produces small amounts of FB_1, FB_2 and FB_3 as well as AAL-toxin.

FB_1 was isolated from a phytotoxic extract of *F. moniliforme* NRRL 18738 and shown to be the compound responsible for phytotoxic symptoms on jimsonweed (1, 3 , 4, 7). FB_1 was confirmed to be the same toxin that caused mammalian toxicity including equine leukoencephalomalacia (52) and porcine pulmonary edema (34).

The structure of FB_1 was determined in 1988 by Bezeidenhout et al., (24) and Gelderblom et al., (36) (Fig. 1). FB_2 and FB_3 lack hydroxyl groups at R_5 and R_3 respectively, and FB_4 lacks hydroxyl groups at both sites (Fig. 1). FA_1 and FA_2 are the N-acetylated forms of FB_1 and FB_2, respectively, and are essentially inactive (Fig. 1). The hydrolysis products, the aminopentols [AP_1 (HFB_1) and AP_2 (HFB_2)], are weakly phytotoxic (6, 75).

FB_1 is also produced by fermentation of *F. moniliforme* on solid media, especially rice (1, 4). The isolate was grown on autoclaved, converted long-grain enriched rice by the following procedure. Fungus-infested rice was transferred to a screen-bottomed tray and allowed to air-dry at room temperature for 72 to 96 h in a ventilated hood. One kg of ground infested rice was soaked in aqueous methanol (40%) at a ratio 1:5 culture to extracting solvent (w/v) overnight at room temperature. Following the soaking, cultures were blended for 5 min at high speed, centrifuged at 10,000 xg for 10 min, and filtered through a double layer of cheese cloth. The filtrates were concentrated in a rotary evaporator at 40° C until all traces of methanol were removed. The water layer was applied to an XAD-2 column for clean-up, followed by a silica gel column. Fumonisin was purified as described in detail by Vesonder et al., (79). Branham and Plattner (26) recently reported that a new fumonisin C_1 has been obtained from a isolate of *F. moniliforme*. This compound lacks the *N*-terminal methyl group (R_1) of FB_1. Nothing is yet known about its toxicity (Fig. 1).

2. Biological Effects

AAL-toxin was first described as the active component of *A. alternata* culture filtrates that cause stem canker disease in *asc/asc* tomatoes (*Lycopersicon esculentum* Mill.) (39). At that time, because heterozygous (*Asc/asc*) and dominant (*Asc/Asc*) genotypes were resistant to AAL-toxin, it was felt that it was a host-specific toxin (33).

When FB_1 was found to be structurally related to AAL-toxin, it was decided to test both toxins for phytotoxicity with a variety of plant species. AAL-toxin was phytotoxic to both weed and crop species, including black nightshade (*Solanum nigrum* L.), redroot pigweed (*Amaranthus retroflexus* L.), northern jointvetch [(*Aeschynomene virginica* (L.) B.S.P.)], duckweed (*Lemna* spp.), prickly sida (*Sida spinosa* L.), jimsonweed (*Datura stramonium* L.), cocklebur (*Helianthus annuus* L.), hemp sesbania (*Sesbania exaltata* [(Raf.)

Table 1. Cytotoxicity of Fumonisin and Analogs with Cultured Mammalian Cell Lines

		IC_{50} (μM)		
Fumonisin Analog	Molecular Weight	MDCK	H4TG	NIH3T3
FB_1	721.84	10	10	150
FB_2	705.81	20	2	—#
FB_3	705.81	50	5	—#
AP_1	405.62	100	—#	100
AP_2	389.59	30	40	35
FA_1	747.89	—*	—*	—*
FA_2	763.86	—*	—*	—*
$NAcAP_1$	447.66	300	150	150
Ac_6AP_1	657.84	400	400	300
1	705.81	100	75	50
2	617.83	300	400	200
3	677.67	400	300	200
4	691.79	100	100	15
5	375.57	100	150	25
6	509.01	300	300	300
7	357.60	25	15	15
8	705.81	15	15	15
9	733.79	200	200	150
Octadecylamine	269.52	—	400	400

*No detectable cytotoxicity at 132 μM
No detectable cytotoxicity at 70 μM.
(Modified table from ref nos. 7 and 71).

Rydb. ex A.W. Hill]), and sicklepod [*Senna obtusifolia* (L.) Irwin and Barneby]. Symptoms included chlorosis, necrosis, stunting and mortality (5, 12).

Symptoms caused by FB_1 on susceptible plants also included chlorosis, necrosis, stunting and mortality. The symptoms were similar to AAL-toxin with redroot pigweed, sunflower (*Helianthus annuus* L.), sicklepod, hemp sesbania, northern jointvetch, soybean [*Glycine max* (L.) Merr.], prickly sida, venice mallow (*Hibiscus trionium* L.), spurred anoda [*Anoda cristata* (L.) Schlecht.], susceptible tomatoes, jimsonweed and black nightshade being susceptible to ≤ 50 μg ml^{-1} (2).

Duckweed (*Lemna pausicostata* L.) has proved to be a sensitive bioassay for AAL-toxin, FB_1 and related compounds (75). Duckweed is a small aquatic plant that can be easily grown in the laboratory (75). Phytotoxic effects can be easily quantified by measuring chlorophyll loss and cellular leakage (74). AAL-toxin was about 10-fold more active than FB_1 in the duckweed bioassay, causing maximal effect at a 0.1 μM concentration. FB_1, FB_2 and FB_3 caused effects identical to those of AAL-toxin in duckweed at 1 μM. The hydrolysis products, AP_1 and AP_2 were much less active, and FA_1 and FA_2 were completely inactive in the duckweed bioassay (75).

FB_1 has been identified as the cause of leukoencephalomalacia, a degenerative brain disorder in horses (52), and pulmonary edema in swine (34). It was first isolated using a short-term assay for tumor promoters (36) and there is continuing concern that it may be a carcinogen (37, 65). Because FB_1 is a mammalian toxin, it is not suitable for use as a herbicide. Therefore, studies on structure-activity relationships for mammalian toxicity of fumonisins have been carried out in mammalian cell cultures in an attempt to find an analog without mammalian toxicity that retains herbicidal activity. NIH3T3 mouse fibroblasts are most resistant to all compounds studied with FB_1, FB_2, and FB_3 causing no significant cytotoxicity at 70 μM (Table 1). However, H4TG rat hepatoma cells were susceptible at low

concentrations (~ 4 μM) of the fumonisins and MDCK dog kidney cells had somewhat higher IC_{50}'s (20 - 56 μM) for the same toxins (7, 71). The most active compound against mammalian cells in our study was FB_2. AAL-toxin was approximately 5-fold less toxic than FB_1 on most cell lines tested. AP_1 and AP_2 showed moderate toxicity against mammalian cell lines (7, 11). No compounds tested had low mammalian toxicity and high phytotoxicity.

3. Mode of Action

Abundant evidence now exists that FB_1, AAL-toxin and their analogs are potential inhibitors of sphingolipid biosynthesis (10, 63, 80, 81, 82). Sphingolipids are essential constituents of cell membranes in both animals and plants (54, 55, 69). Most of the research on the mode of action of this class of natural products has been carried out with FB_1 in mammalian systems. FB_1 has been shown to inhibit the enzyme sphinganine (sphingosine) N-acyl-transferase (ceramide synthase) (81, 82). Elevated levels of free sphinganine and the sphinganine: sphingosine ratio are observed in animal cells treated with FB_1 (64), and in the serum and tissue of animals exposed to dietary fumonisins (63, 80). It has been hypothesized that disruption of sphingolipid metabolism is a contributing factor in the animal diseases associated with consumption of fumonisin contaminated feeds and foods.

Sphingosine and sphingosine 1-phosphate can induce DNA synthesis in growth-arrested Swiss 3T3 cells. FB_1 incubated with the cells elevated sphingosine and induced an increase in [^{3}H] thymidine incorporation into DNA (59). Further studies showed that sphinganine was required for stimulation of DNA synthesis. Studies with LLC-PK renal cells showed a close correlation between fumonisin-induced cytotoxicity and inhibition of *de novo* sphingolipid biosyntheses (82). However, studies with primary rat hepatocytes have not shown any relationship between cytotoxicity and elevation of free sphingoid bases (38).

Our work has focused on confirmation of a similar mode of action in plant tissues (10). Following treatment with FB_1 or AAL-toxin, the free sphingoid bases, phytosphingosine and sphinganine, were found to be markedly elevated relative to controls in the duckweed bioassay (Table 2). Extracts of treated tomato plants and tobacco callus cultures showed the same results. FB_1 and AAL-toxin caused 18- and 45-fold increases in phytosphingosine and 76- and 129-fold increases in sphinganine, respectively, after 24 h (10).

Phytosphingosine and sphingosine also have phytotoxic effects on duckweed at higher concentrations (50 μM) causing increased conductivity and chlorophyll loss (75). This suggests that the build-up of free sphingoid bases is toxic to cells (65, 75). The

Table 2. Concentration (pmol/mg dry weight) of free phytosphingosine and free sphinganine in duckweed treated with 1 mM fumonisin B_1 or AAL-toxin; and susceptible tomato leaf discs (LA12) with 1 mM AAL-toxin for 24 h

Treatment	Phytosphingosine	Sphinganine
Duckweed		
Control	17	6
Fumonisin B_1	309	454
AAL-toxin	770	776
Tomato leaf discs		
Control	7	4
AAL-toxin	279	1749

Values are means from three to six independent experiments. (Modified table from Ref. no. 10).

mechanisms of cellular toxicity of free sphingoid is unknown, but they do not appear to be acting as a cationic detergent (75).

The fumonisins have been associated with human cancer (32, 77), although the mechanism of carcinogenicity is unknown. FB_1 was not found to be mutagenic in the *Salmonella* mutagenicity assay and was not genotoxic in DNA repair assays (65). The fumonisins appear to act mainly as tumor promoters.

It is unknown how the elevated levels of free sphingoid bases caused by FB_1 and AAL-toxin in plants might mediate the physiological changes observed. It is possible that changes in membrane permeability are involved. The structural analogy with sphingosine

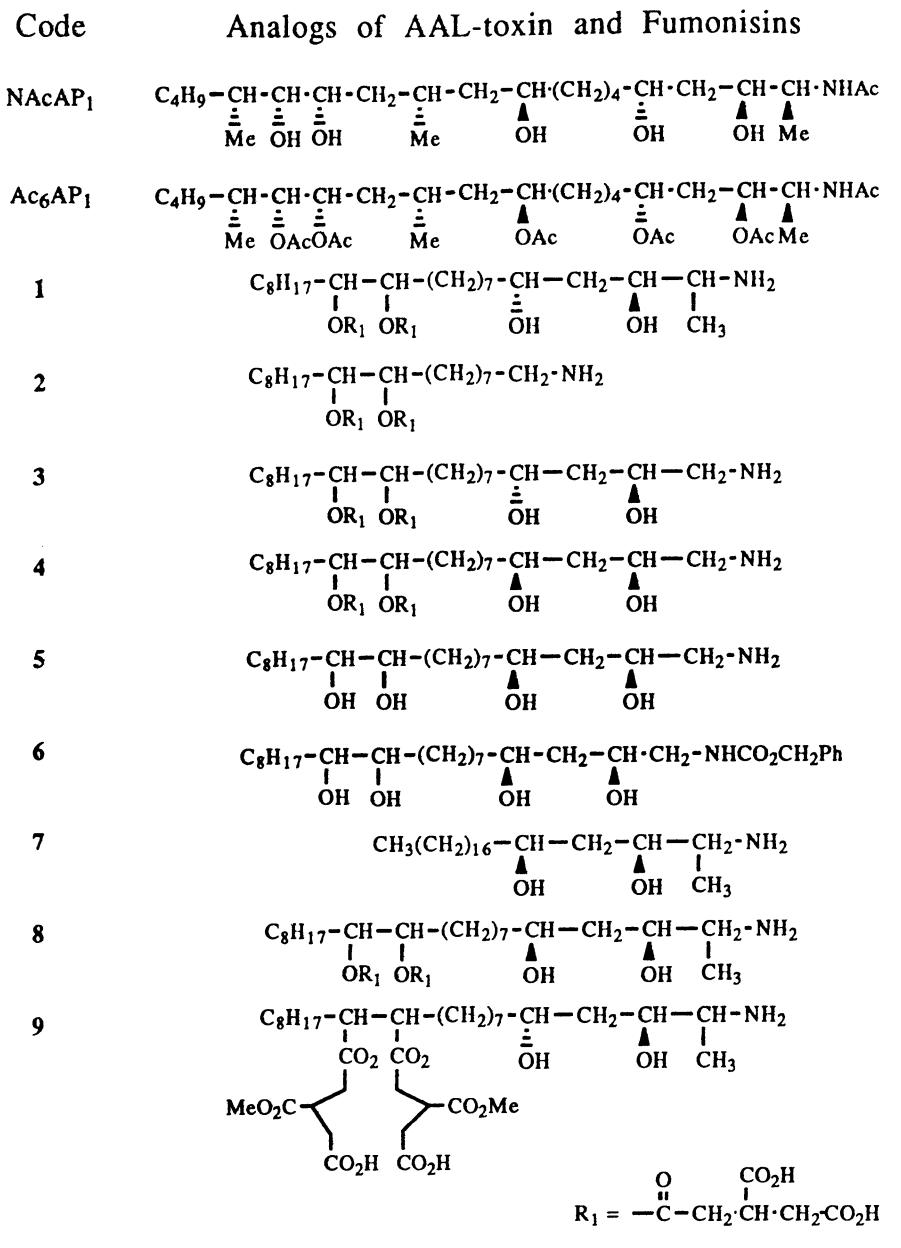


Figure 2. Chemical structure of various synthetic analogs of AAL-toxin and fumonisins.

also suggests an alternate mechanism of action for FB_1 and AAL-toxins. FB_1, AAL-toxin and notably the hydrolyzed toxins are structural analogs of all sphingolipids, but they are particularly closely analogous to sphingosine and sphinganine, a biosynthetic intermediate in the *de novo* synthesis of sphingolipids. Both sphingosine and sphingolipids play key roles in cells. Sphingosine is believed to be an important intracellular regulatory molecule (41) which has been hypothesized to be produced in cells by effector-stimulated degradation of sphingolipids. Sphingosine and FB_1 have been shown to inhibit the activity of protein kinases, notably protein kinase C (42, 46), a key regulatory enzyme which is activated by the well-studied tumor promoter, phorbol myristate acetate (29). The full extent of sphingosine's ability to regulate other protein kinases, such as MAP kinases that are thought to be important in the regulation of cell proliferation and the mechanism of tumor promotion, remains to be determined (54). Sphingolipids are essential structural components of cell membranes; for example, selective destruction of sphingolipids by sphingomyelinases such as staphylococcal ß-toxin is a very effective method for destroying membrane integrity (35). Therefore, disruption of sphingolipid metabolism could alter many biochemical processes essential to the normal function and viability of cells.

4. Analogs

A basic requirement for a safe herbicide is that it exhibit strong phytotoxicity and weak or no mammalian toxicity. The demonstration that FB_1 is a tumor promoter eliminates it from direct consideration as a herbicide. However, the finding that FB_1 is phytotoxic by a different mechanism than any commercially used herbicide makes it worthwhile to search for analogs with low mammalian toxicity and high phytotoxicity.

The fumonisin and AAL-toxin analogs were obtained from a variety of sources. Aminopentols, AP_1 and AP_2 were either the gifts of W.C.A. Gelderblom (7) prepared by hydrolysis of purified FB_1 and FB_2, respectively, or prepared in our laboratory in larger quantities by hydrolysis of crude fumonisins with subsequent purification of the aminopentols (70). The latter approach is somewhat simpler because it avoids the difficulties involved in purifying zwitterions. An extensive series of analogs (Fig. 2) was prepared from oleic acid (17, 49, 50, 74). These analogs were tested on duckweed and susceptible tomato leaf discs for phytotoxicity and on three mammalian cell lines for mammalian toxicity (11, 13).

The most notable thing about these results is that a wide range of structures retains biological activity in both the plant and mammalian systems. Of the compounds tested, analog 9 has the best combination of high phytotoxicity and low mammalian toxicity. The basis for the selectivity may simply reflect higher levels of non-specific carboxylesterase activity in plant tissues. Other analogs of the fumonisins and AAL-toxin will continue to be investigated for desirable characteristics.

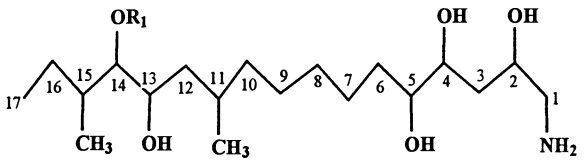
5. Biosynthesis and Metabolism of AAL-Toxin and Fumonisins

The substantial structural analogy between fumonisins, AAL-toxin, sphingosine, and related sphingolipids was recognized early in the studies on the toxins, and it has shaped people's ideas about mode of action and probable biosynthetic and metabolic pathways.

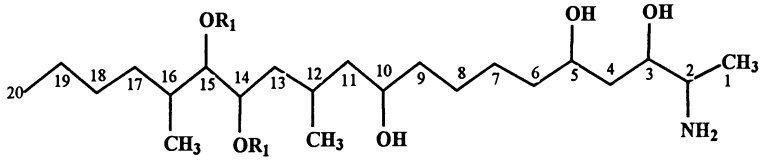
Sphingolipids are important constituents of cell membranes in animals, plants, fungi and some bacteria (78). Some important long-chain sphingoid bases are shown in Figure 3.

The biosynthesis of sphingolipids has been studied in mammalian systems (47) and in yeast (73). They are constructed by condensation of the co-enzyme A derivative of a long chain fatty acid, usually palmitate (C-16) with serine with concomitant loss of the carboxyl group of serine. The ketone group is then reduced to give sphinganine, a close structural

AAL-TOXIN

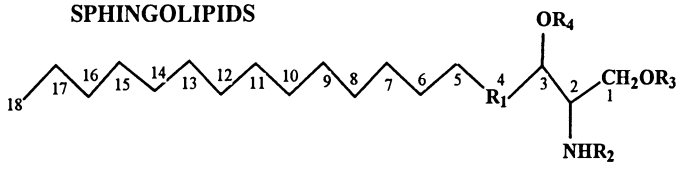


FUMONISIN B_1



R_1 = CO-CH_2-CH(CO_2H)CH_2CO_2H

SPHINGOLIPIDS



Sphingolipid	R_1	R_2	R_3	R_4
Sphingosine	CH=CH	H	H	H
Phytosphingosine	CH_2-CHOH	H	H	H
Sphinganine	CH_2-CH_2	H	H	H
Tetraacetyl-phytosphingosine	CH_2.CH(O-CO-CH_3)	CO-CH_3	CO-CH_3	CO-CH_3
N-Lignoceroyl-D,L-sphinganine	CH_2-CH_2	CO-$(CH_2)_{22}$-CH_3	H	H

Figure 3. Chemical structure of various sphingolipids.

analog of the fumonisins. It is subsequently acylated on the amino group, and a double bond introduced at the carbon adjacent to the hydroxyl group.

All evidence so far obtained indicates that fumonisin is biosynthesized by a pathway analogous to sphingosine rather than the acetogenin pathway that is used in the biosynthesis of aflatoxins and many antibiotics from closely-related *Streptomycetes* and fungi. We have observed (15) that propionate, which is predicted to be a good precursor by the acetogenin pathway, is not an effective precursor of FB_1, whereas methionine, which is predicted to be a good precursor in the sphingosine-type pathway, has been observed to be an excellent precursor of FB_1 in our studies (15) and the work of others (16, 20, 21, 61). Similarly, we (15) and others (27) have observed that alanine is a good precursor of fumonisins. It is predicated to be the precursor of C(1) and C(2) as well as entering the side chains of fumonisins and AAL-toxins via the citric acid cycle (Fig. 4).

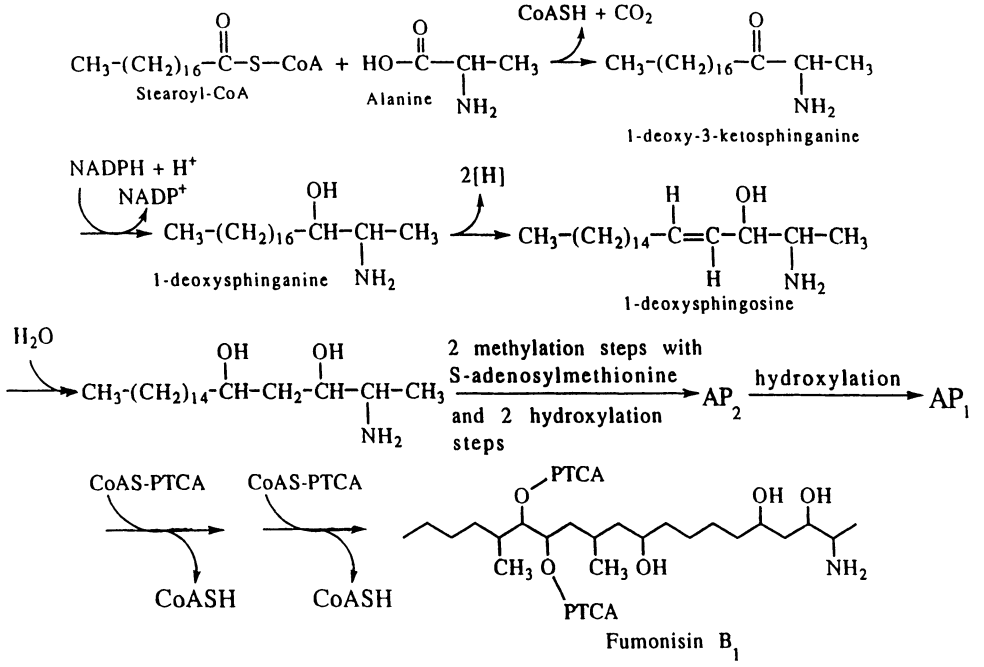


Figure 4. Proposed biosynthetic pathway for fumonisins.

Structural analogy to sphingosine has also shaped thinking about the metabolism of fumonisins. The major metabolic pathway for sphingosine in mammalian tissues occurs by phosphorylation on the hydroxyl of C(1) followed by a retro-aldol reaction which cleaves off a small fragment containing the amino group (53). Deviations from the sphingosine structure in both fumonisins and AAL-toxins block this metabolic pathway because the toxins lack the hydroxyl on C(1), and the result is substantially enhanced metabolic stability relative to sphingosine. Indeed, metabolic stability is the only thing that has been observed experimentally in studies of fumonisin metabolism. Radio-labelled fumonisin B_1, whether it is administered orally or by injection, is almost entirely eliminated unmetabolized in the feces of rats (51, 67) and primates (68), suggesting that it is being excreted in the bile. Cultured rat hepatocytes did not yield readily detected metabolites with radiolabelled FB_1 either (30). It must be concluded that either FB_1 exerts its numerous biological activities in an unmetabolized form, or there are highly active metabolite(s) formed in amounts too small to be detected by the methods used (Fig. 5).

Structural analogy with sphingosine prompts a predicted metabolic pathway for FB_1. In Fig. 5 it is predicted that FB_1 may be (i) acylated by ceramide synthetase with or without removal of the side chains by carboxylesterase action; and (ii) de-aminated by either monoamine oxidases or mixed function oxidases with or without esterase action to yield metabolites that would be expected to be inactive, since a free amino group appears to be required for activity. However, none of the metabolites predicted in Fig. 5 are likely to be highly active.

6. Absolute Stereochemistry of AAL-Toxin and Fumonisins

The 2D structure of AAL-toxin was determined by Bottini et al., (22, 23) in 1981 and of fumonisin B_1 and B_2 by Bezuidenhout et al., (24) in 1988, but the 3D structure has only

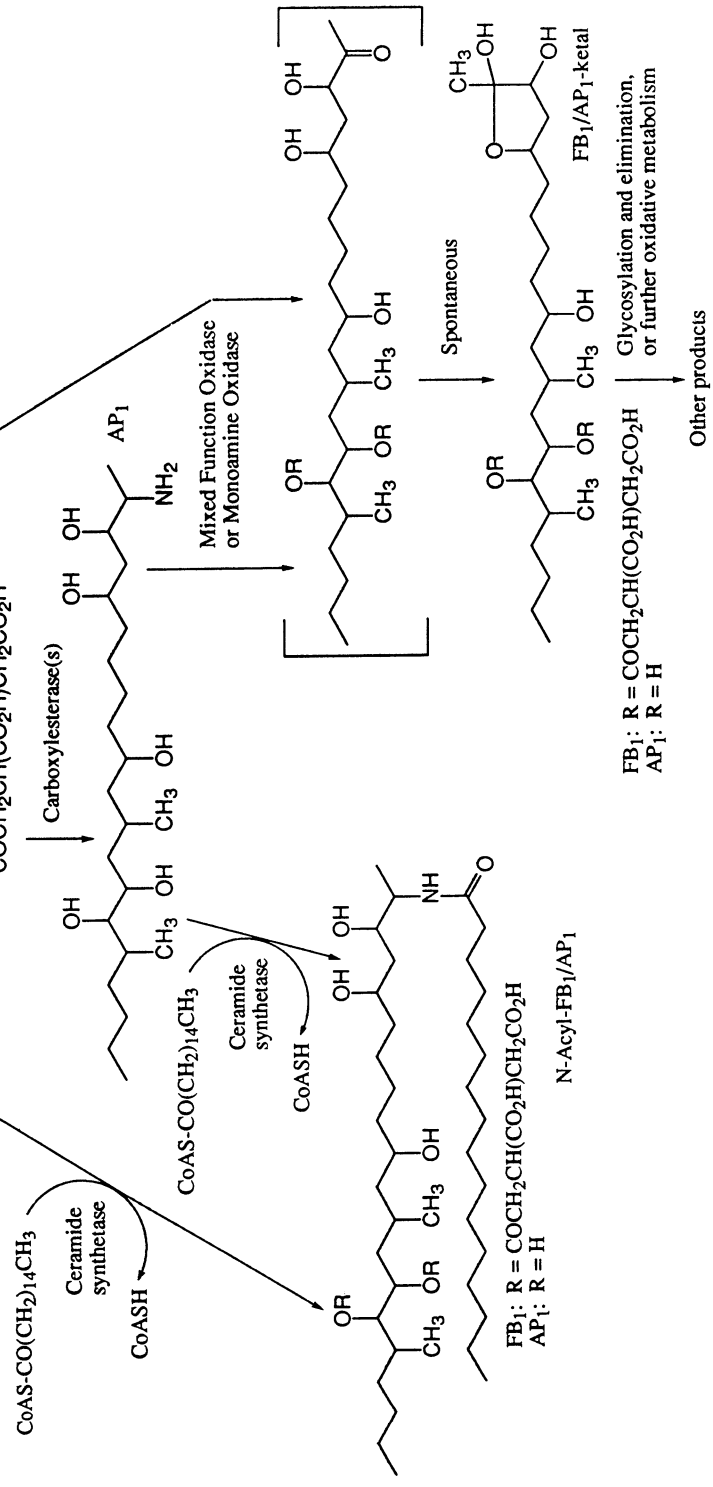


Figure 5. Predicted metabolic pathway of fumonisins.

recently been determined through the contributions of several research groups. The stereochemistry was determined in order to complete the structure determination, and because it can provide information about the biosynthesis of the toxins. The presence of a single configuration at a stereogenic carbon implies the presence of an enzyme to catalyze its introduction. The study of FB_1 backbone stereochemistry has provided a particularly useful piece of information about its biosynthesis in the case of the C(2) configuration. The configuration at C(2) was found to be the opposite to that of the C(2) in sphingolipids in four independent studies using three different methods (i) synthesis (43); (ii) NMR analysis of a ring derivative (18, 19, 62); and (iii) Mosher amide analysis (45). This observation makes it unlikely that the enzyme which couples stearoyl-CoA to alanine in FB_1 biosynthesis or palmitoyl-CoA to glycine in AAL-toxin biosynthesis is closely related to the corresponding sphingolipid biosynthesis enzyme which couples palmitoyl-CoA to serine.

The various research groups that have contributed to the understanding of fumonisin and AAL-toxin stereochemistry have used a variety of approaches. The approach used by Boyle et al., (25) and Oikawa et al., (58) has been to synthesize model compounds representing fragments of the toxins in all possible configurations and assign the correct one by detailed comparison of the NMR spectra. Pock et al., (62) and ApSimon et al., (18, 19), as well as ourselves (45) have made extensive use of NMR analysis to determine relative configurations, particularly using derivatives which lock two or more stereogenic centers into rings. Our group has also made extensive use of two other techniques, Mosher ester analysis (45) and chiral column chromatographic analysis of suitably derivatized fragments to provide information on the absolute configurations. The latter technique has recently been used to complete the determination of the absolute configuration of the last remaining unknown stereogenic centers in FB_1 and AAL-toxin (72). Because propane-1,2,3-tricarboxylic acid is a symmetrical molecule it was necessary to use a reagent that would selectively reduce either the ester or the free carboxylic acids while the side chains were still attached to the backbone. Selective reduction of the free carboxylic acids to alcohols with diborane was chosen. The alternative approach, selective reduction of the ester linkage with sodium borohydride, was not chosen because the alkaline conditions used with this reagent might cause racemization of the stereogenic centers. Several conversion steps were required after release of the side chains in order to convert them into a form which would separate on the chiral column. As shown in Fig. 6, the side chains were converted to 3-methylvaleric acid methyl ester, the racemic form of which could be resolved on a chiraldex GT-A chiral gas chromatography column. Identification of the absolute configuration was made by comparison of retention times with authentic (S)-3-methylvaleric acid methyl ester prepared by deamination of L-isoleucine, which has a known absolute configuration from x-ray crystallography studies. These results combined with previously reported studies on the FB_1 backbone in our group (44) and on the AAL-toxin backbone by Boyle et al., (25) completes the determination of the 3D structures of both toxins (Fig. 6).

7. Conclusions

We have reviewed a variety of aspects of the chemistry and biological activities of AAL-toxin and the fumonisins. Both AAL-toxin and the fumonisins are toxic to a wide range of weed and crop species. Investigation of the mode of action of this class of toxins in plant tissues demonstrates that an early effect is disruption of sphingolipid metabolism. FB_1 and AAL-toxin cause buildup of phytosphingosine and sphinganine, most probably due to inhibition of ceramide synthesis. This mechanism appears to be the same as that described in animals for FB_1. Thirteen analogs of AAL-toxin and FB_1 were obtained and tested for phytotoxicity and mammalian cell culture toxicity. Analog 9, a diester derivative, caused phytotoxic effects in leaf disc and duckweed bioassays, while showing less toxicity to

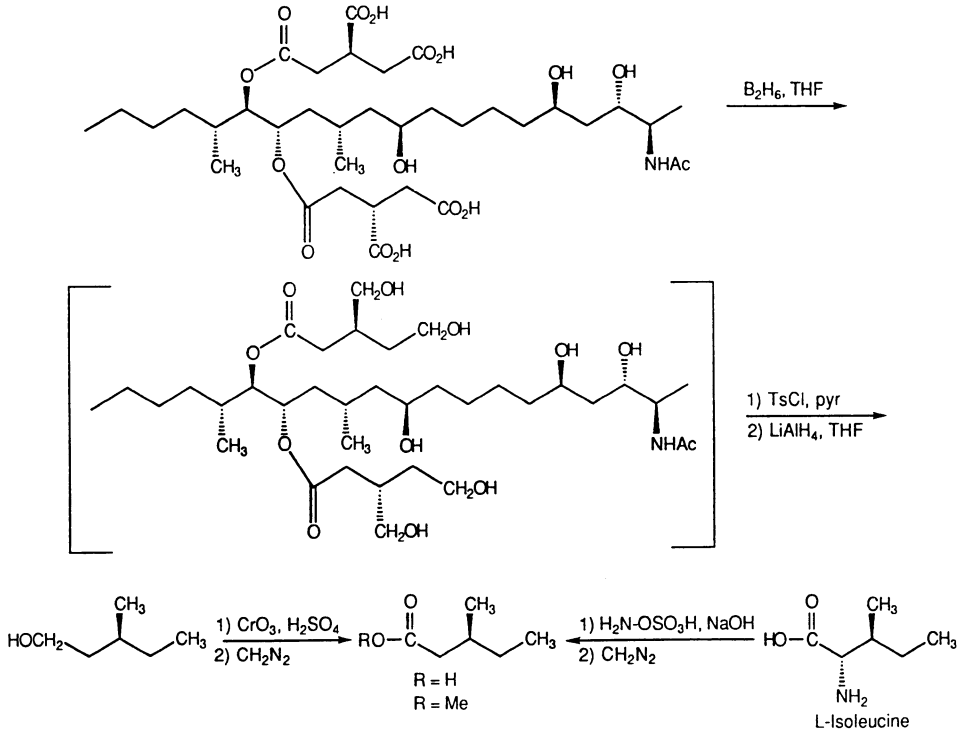


Figure 6. Determination of the absolute configuration of the side chains of fumonisin B_1. AAL-toxin was studied in the same manner.

mammalian cells than FB_1. AAL-toxin and the fumonisins are themselves sphingosine analogs. As such, this has helped us understand better the biosynthesis and metabolism of these toxins. Also, the role of sphingolipids in membrane integrity suggests the molecular basis of phytotoxicity. Finally, we can now state with confidence the absolute configuration of AAL-toxin and the fumonisins. This structure also suggests differences in the biosynthetic pathways of the toxins and sphingolipids. All these studies have contributed to our knowledge of AAL-toxins and the fumonisins especially with regard to their phytotoxicity. It is hoped that these studies will eventually lead to possible use of analogs of these compounds in weed control.

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