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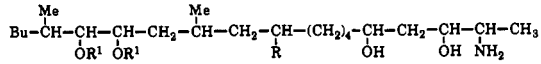
SHORT COMMUNICATIONS

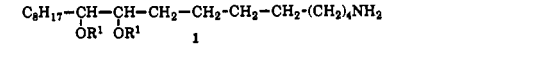
Synthesis of Analogs of Fumonisin B_1

The fungus *Fusarium moniliforme* Sheldon is one of the most important ear rot pathogens of corn (Nyvall, 1983). The toxicity of the mold was initially discovered when an isolate of *F. moniliforme* from corn grown in an area of Africa with a high incidence of esophageal cancer was tested (Marasas et al., 1981). This isolate induced quine leukoencephalmalacia in horses (Kriek et al., 1981), was hepatocarcinogenic to rats (Jaskiewicz et al., 1987), and was mutagenic to *Salmonekla typhimurium* (Gelderblom et al., 1986). Fumonisins are considered tumor promoters because their culture material induces γ-glutamyltransaminase positive foci in rat liver, which is a well-established bioassay for tumor promoters (Norred et al., 1992). Fumonisins are also known to be inhibitors of sphingosine biosynthesis (Wang et al., 1991). The isolation and structural elucidation of the fumonisins have been published (Bezvidenhout et al., 1988; Plattner, 1992).

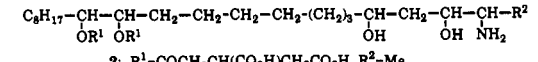
The stereochemistry of the fumonisins is unknown; therefore, the synthesis of analogs became our initial goal. Analogs of fumonisin which exhibit fumonisin activity will enable toxicologists to study the dependence of activity on structure. Determination of the minimum structural requirements for activity may enable researchers to prepare inhibitors of fumonisins. *Our hypothesis was that the tricarballylic side chains and the amine constituted the minimum functional requisites for activity.* Our synthetic objectives therefore became analogs 1–3. No



FB_1: R^1 = $COCH_2CH(CO_2H)CH_2CO_2H$, R = OH
FB_2: R^1 = $COCH_2CH(CO_2H)CH_2CO_2H$, R = H



1



2: R^1=$COCH_2CH(CO_2H)CH_2CO_2H$, R^2=Me
3: R^1=$COCH_2CH(CO_2H)CH_2CO_2H$, R^2=H

synthetic method for the introduction of the tricarballylic side chain had been published. Our approach to 1 began with oleylamine (4). Oleylamine was reacted with benzyl chloroformate and sodium carbonate (Bergmann and Zervas, 1932) to yield carbamate **5** in 75% yield. After a number of unsuccessful attempts to introduce the side chains, the Prevost reaction was examined (Wilson, 1957). The Prevost reaction (Scheme I) involves the oxidation of alkenes with iodine and silver carboxylates. Under anhydrous Prevost conditions, the reaction with cyclohexene yields the *trans*-diacyl derivative. However, the *cis*-diacyl derivative can be obtained when the reaction is conducted in the presence of water. The silver salt **6** (Brice and Simons, 1951) was quantitatively generated by reacting tricarballylic anhydride **7** (Emergy, 1891) with silver oxide in the absence of light. The reaction of *trans*-2-butene and **6** under the Prevost conditions yielded 80% of the anhydride **8**. Carbamate **5** was then reacted with 2 equiv of **6** in the presence of 1 equiv of iodine in boiling benzene for 2 days to produce the bis(anhydride) **9** in 90% yield. Hydrolysis of anhydride **9** with 10:1 THF/water and a catalytic amount of CF_3CO_2H at 25 °C afforded the tetraacid **10** in quantitative yield. The removal of the *N*-benzyloxycarbonyl protecting group was not straightforward. Although hydrogenation with palladium on carbon (Pd/C) in ethanol/water and the use of a hydrogen-transfer reagent such as cyclohexene failed, the hydrogenation of **10** with PtO_2, Pd/C, trifluoroacetic acid, and acetic acid (Hays et al., 1991) afforded a 90% yield of analog **1**. Purification of **1** was effectd by high-performance liquid chromatography (HPLC) on a C_{18} column.

The synthesis of analogs **2** and **3** began with ketone **11** (Tsuji and Hashiguchi, 1981). Reaction of the enolate of **11** (formed by the reaction of **11** with 1.2 equiv of LDA in THF) with aldehydes **12** and **13** (Scheme II) (Stammer and Khatri, 1979) at −78 °C afforded the β-hydroxy ketones **14a** and **14b** in 54 and 45% yields, respectively. Unfortunately, the aldol condensation with **12** showed low diastereoselectivity. The reaction of **14a,b** with $Me_4NBH(OAc)_3$ (Evans and Chapman, 1986) in acetonitrile/acetic acid at 0 °C for 2 days produced diols **15a** and **15b** in 70 and 63% yields, respectively. The final steps of the synthesis of analogs **2** and **3** are the same as those for analog **1**. The diols **15a,b** were heated with the silver salt **6** in benzene providing 70% of **16a** and 64% of **16b**. Hydrolysis of **16a,b** with THF/water/trifluoroacetic acid

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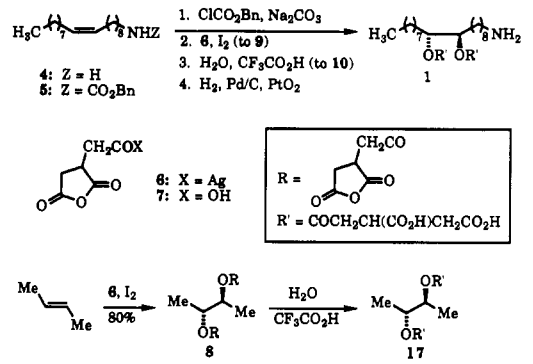
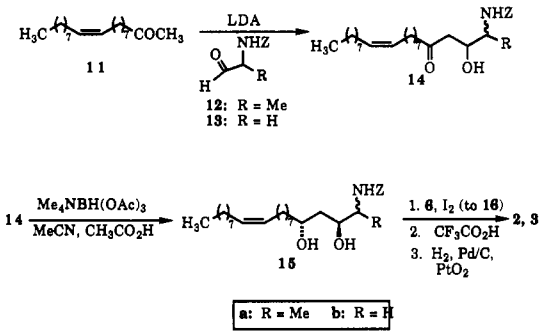
Scheme I



Scheme II



followed by hydrogenation with PtO_2, Pd/C, trifluoroacetic acid, and acetic acid yielded analogs **2** and **3**. Purification of **2** and **3** was effected by HPLC. To determine more about the structural requirements for fumonisin activity, anhydride **8** was hydrolyzed to produce tetraacid **17**.

Toxicity studies using in vitro assays was used to evaluate the comparative toxicity of analogs **1–3** and **17** to FB_1. In this assay all analogs and FB_1 were dissolved in dimethyl sulfoxide (DMSO) and incorporated into the cell culture media. The level of DMSO in any one culture did not exceed 1% of the total volume of media. Cells from a continuous cell line derived from rhesus monkey kidney cells (MA104) were grown to confluency in 96-well tissue culture plates. The growth medium [10% Serum Plus (JRH Biosciences, Lenexa, KS) in Dulbecco's modified Eagle's medium (DMEM) (Gibco Labs, Grand Island, NY)] was removed and replaced with maintenance medium (2% Serum Plus in DMEM) containing various levels of FB_1 or fumonisin analogs. Following 48 h of incubation at 37 °C and 5% CO_2 atmospheric conditions, the cultures were evaluated for cell viability. The number of viable cells was determined by the addition of the tetrazolium dye MTT. Analogs **2** and **3** were more toxic than FB_1, and analog **1** was comparable in toxicity to FB_1. Analog **17**, which contains only the tricarballylic side chain unit, was not toxic, even at levels of 250 ppm.

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