glucopyranoside of medicagenic acid by unambiguous means. Since only indirect evidence supported the original assignment of the 3-O substitution over the 2-O substitution in compound II (Morris and Tankersley, 1963), additional ^{13}C NMR study was undertaken (Table II). The shift of C-3 from δ 76.01 to 86.10 with only a minor change at C-2 supports 3-O substitution, and the peak at δ 105.45 is a further illustration of the β anomeric linkage in compound II.

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Registry No. I, 599-07-5; II, 49792-23-6; III, 5159-36-4; IV, 104693-46-1; V, 22471-97-2.

Supplementary Material Available: Melting points (Table I) and spectral data for compounds I and III–V (2 pages). Ordering information is given on any current masthead page.

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Production of Fusarin C by *Fusarium spp.*

Jeffrey M. Farber* and Gregory W. Sanders

Various Canadian species of *Fusarium* were studied for their ability to produce fusarin C. On corn, all 10 *Fusarium graminearum* strains produced fusarin C at levels much higher than those reported for South African strains. Besides *F. graminearum*, five other species also produced the mycotoxin (*Fusarium avenaceum, Fusarium culmorum, Fusarium poae, Fusarium sambucinum, Fusarium sporotrichioides*). Experiments done with *F. graminearum* growing in liquid culture demonstrated that aeration, temperature, and pH played critical roles in the biosynthesis of fusarin C. Optimal production occurred in 100 mL of glucose–yeast extract–peptone medium (pH 6.0), which was shaken at 100 rpm and incubated at 28 °C.

INTRODUCTION

Fusarin C, a highly mutagenic and potentially carcinogenic compound (Gelderblom et al., 1984a,b; Marasas et al., 1984a; Cheng et al., 1985), was first isolated from extracts of a North American strain of *Fusarium moniliforme* Sheldon growing on cracked corn (Wiebe and Bjeldanes, 1981). This mycotoxin was later found to be produced as well by South African strains of *F. moniliforme* (Gelderblom et al., 1983, 1984b). The structure of the compound has been elucidated (Figure 1; Gelderblom et al., 1984a; Gaddamidi et al., 1985) and its biosynthetic pathway partially worked out (Steyn and Vleggaar, 1985).

Fusarin C has been found to occur naturally in both hand-selected visibly *Fusarium*-infected and healthy-looking corn kernels in South Africa (Gelderblom, 1984b)

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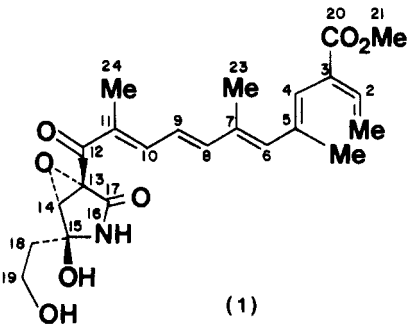

Figure 1. Structure of fusarin C (1).

and in corn collected in Linxian county, China (Cheng et al., 1985). Twelve samples of Ontario corn that were contaminated with deoxynivalenol did not contain detectable levels of fusarin C, although 1.5 μg/g was found in unsterilized field corn incubated in the laboratory (Scott et al., 1985).

Recently (Farber and Sanders, 1986), a liquid culture medium was developed to screen North American isolates of *F. moniliforme* for fusarin C production. Several Canadian isolates produced the toxin in large amounts while growing on corn. Since *Fusarium graminearum* can produce fusarin C (Gelderblom et al., 1984b) and because the organism is an important pathogen of cereal crops in Canada, its ability to produce fusarin C in liquid culture and on corn was investigated. Additionally the ability of other Canadian species of *Fusarium* to produce fusarin C was further studied.

MATERIALS AND METHODS

Organisms. *Fusarium culmorum* strains F2, F3, and F7, *Fusarium poae* strain 1133/28A, and *Fusarium sporotrichioides* strains 1065/30B and 1136/17B were obtained from Dr. A. V. Sturz, Agriculture Canada, Charlottetown, Prince Edward Island. Fungi were obtained from the International Toxic *Fusarium* Reference Collection (ITFRC) University Park, PA (*Fusarium solani* S-735, *Fusarium semitectum* R-6355, *Fusarium avenaceum* R-4608) and the ATCC (*F. culmorum* 34913). The remaining two isolates of *F. solani* (HPB10 and 11) were cultures maintained in our own collection. All of the other *Fusarium* isolates were obtained from Agriculture Canada, Department of Agriculture Ottawa Mycology (DAOM) Collection, Ottawa, Ontario.

Media. GYEP (glucose 10 g, yeast extract 1 g, peptone 1 g; Ueno et al., 1975), Czapek-Dox (Blight and Grove, 1974), and MYRO (Brewer et al., 1982) medium were initially used to induce fusarin C production by *F. graminearum* in liquid culture. To test the ability of *Fusarium* isolates to grow and produce fusarin C on a solid substrate, 50 g of whole-grain corn kernels plus 20 mL of distilled water were added to 250-mL Erlenmeyer flasks, which were autoclaved and then inoculated.

Inoculation Procedure. Corn cultures were inoculated as described previously (Farber and Sanders, 1986). Duplicate corn cultures were inoculated and incubated for 21 days at 28 °C in an atmosphere of 70% relative humidity. All flasks that were not analyzed immediately were frozen at −20 °C.

To inoculate liquid cultures, the inoculation medium (Farber and Sanders, 1986) after incubation at 28 °C for 48 h was centrifuged (10240*g*, 30 min) and the cells resuspended in sterile distilled water. Aliquots (two 5-mL portions) of the cell suspension were dried to a constant weight and the cell concentration subsequently adjusted to the desired level for each experiment. Triplicate flasks containing liquid media were inoculated and incubated at 28 °C for 7 days (shaking at 100 rpm) unless otherwise indicated. *F. graminearum* 180379 was the strain used in all liquid culture experiments. All analytical procedures were performed under "gold" fluorescent lighting (Westinghouse 6YT 126D) due to the known sensitivity of fusarin C to long-wave UV light (Gelderblom et al., 1983).

Extraction Procedures. *I. Liquid Culture.* These media were extracted and prepared for analysis as previously described (Farber and Sanders, 1986).

II. Corn Cultures. From 50 g of corn, 10-g portions were removed and blended (Kenmore 16 blender; Simpson Sears Ltd., Toronto, Canada) for 5 min in 100% CH_3CN (Scott et al., 1985). The blended material was passed through a Buchner sintered-glass funnel (150-mL capacity), evaporated to dryness under vacuum at 30 °C, and resuspended in 2.0 mL of 3% CH_3OH in CH_2Cl_2. A suspension (0.5 mL) was then applied to a 3-mL disposable silica gel column (Baker 10 SPE, J. T. Baker Chemical Co., Phillipsburg, NJ), which was eluted with about 2.8 mL of 10% CH_3OH in CH_2Cl_2. The eluant was evaporated to dryness under a gentle stream of nitrogen and reconstituted in 0.5 mL of $CHCl_3$. Depending on the analyses, 5–50 μL was transferred into 0.5-dram vials with Teflon-lined caps, evaporated to dryness under a stream of nitrogen, and stored at −20 °C until analyzed.

Fusarin C Analyses. TLC and HPLC analyses to confirm the presence or absence of fusarin C were performed as described previously (Farber and Sanders, 1986). Mass spectrometry was done on TLC-purified extracts obtained from *F. graminearum* 180379 growing in GYEP medium and individually on several pooled extracts from various *F. graminearum* and *F. culmorum* strains growing on corn, to confirm the presence of fusarin C (mass spectrometric M^+ m/e 431). A VG Micromass ZAB-2F mass spectrometer operated at 1.5K resolution, 70-eV electron energy, and 200 °C ion source temperature was used. For all other *Fusarium spp.*, fusarin C production was confirmed by comparing the HPLC chromatograms of irradiated (long-wave UV light, 1 min) purified fusarin C with irradiated sample extracts (Gelderblom et al., 1984b).

Liquid Culture Experiments. (*i*) *pH.* The pH of GYEP medium was adjusted with either 1 N NaOH or 1 N HCl to observe effects on fusarin C production. The initial pH of GYEP medium for all other experiments was 6.0–6.1.

(*ii*) *Aeration.* The effects of various aeration conditions on growth and toxin production by *F. graminearum* 180379 was investigated. Inoculated flasks incubated in Model G-25 incubator shakers (New Brunswick Scientific Co., Inc., Edison, NJ) were shaken at either 50, 100, 150, or 220 rpm. In the remaining experiments 100 rpm was used.

(*iii*) *Temperature.* Flasks containing 50 mL of GYEP medium were inoculated with 2.5 mg of dry wt/50 mL of cell suspension and incubated at 20, 25, 28, and 32 °C. In all other trials 28 °C was the temperature of incubation.

(*iv*) *Sugar Concentration.* The glucose concentrations in GYEP medium were varied from 5 to 40 g/L to observe the effect on toxin production. For all other trials the concentration was 10 g/L.

(*v*) *Different Volume Experiment.* The volume of GYEP medium added to the 250-mL Erlenmeyer flasks was varied from 25 to 100 mL to observe the effect on fusarin C production. For all other experiments 50 mL of medium in a 250-mL Erlenmeyer flask was used.

(*vi*) *Inoculum.* The effect of varying the initial inoculum was tested. The amounts of cell material inoculated were

Table I. Effect of Aeration and Culture Volume on the Production of Fusarin C by *F. graminearum* in GYEP

aeration conditions,[a] rpm	fusarin C,[b] μg/L	culture vol[d]	fusarin C,[b] μg/L
nonshaking	1710	25	3370
50	1160	50	6330
100	8500	75	16050
150	ND[c]	100	23770
220	ND		

[a] pH 6.0; 2.5 mg of dry wt cells/50 mL of medium; 28 °C. [b] Values are means of triplicate determinations. [c] ND not detected. [d] As (a) above but at 100 rpm.

Table II. Effect of Temperature and Initial pH on the Production of Fusarin C by *F. graminearum* in GYEP

temp,[a] °C	fusarin C,[b] μg/L	init pH[c]	final pH	fusarin C,[b] μg/L
20	138.5	7.0	6.1	160
25	2510	6.5	6.4	2520
28	7700	6.0	6.1	5080
32	10	5.5	5.8	1820
		5.0	5.3	1220

[a] pH 6.0; 2.5 mg of dry wt cells/50 mL of medium; 100 rpm. [b] Values are means of triplicate determinations. [c] As in (a) above except experiments were performed at 28 °C and at stated pH values.

2.5, 5.0, 10, 25, and 50 mg of dry wt/50 mL of cell suspension. In all other cases 2.5 mg of dry wt/50 mL of liquid medium was used.

RESULTS AND DISCUSSION

Initial experiments performed with *F. graminearum* isolates growing in GYEP, Czapek-Dox, and MYRO media (both shaking at 220 rpm and nonshaking) demonstrated that only GYEP nonshaking was able to support fusarin C production. Later attempts to optimize production of the compound showed that GYEP medium shaking at 100 rpm supported maximal production of the mycotoxin (Table I). This contrasts with production of fusarin C predominantly in MYRO medium (220 rpm) by *F. moniliforme* (Farber and Sanders, 1986).

When the inoculum was increased from 2.5 mg of dry wt cells/50 mL of media to 7.5 or 10.0 mg, no fusarin C could be detected (by HPLC). The largest concentration of fusarin C in liquid culture medium (23.8 ppm) occurred in flasks containing 100 mL of liquid culture (Table I).

These observations (Table I) suggest that under conditions that led to decreased amounts of O_2, greater amounts of fusarin C were produced. Thus, as for deoxynivalenol (Miller et al., 1983) and aflatoxin (Shih and Marth, 1974), one of the stimuli for fusarin C production by *F. graminearum* may be lowered O_2 concentration.

The optimum temperature for production of fusarin C by *F. graminearum* (in GYEP medium) was 28 °C (Table II). Above this temperature, a precipitous drop in fusarin C production occurred, a phenomenon not observed with a producing culture of *F. moniliforme* growing in liquid (MYRO) medium (unpublished results). Although other investigators have reported optimal *Fusarium* mycotoxin production in liquid culture at 28 °C (Miller et al., 1983), compounds such as T-2 toxin, diacetoxyscirpenol, and zearalenone have been found to be produced in greater amounts at lower (15–20 °C) temperatures (El-Kady and El-Maraghy, 1982; Vidal et al., 1984; Bergers et al., 1985).

The optimum pH for fusarin C production in liquid medium was approximately 6.0–6.1 (Table II). This contrasts with the optimum pH value (around 3.0) for biosynthesis of the same mycotoxin by *F. moniliforme* in MYRO medium (Farber and Sanders, 1986). The importance of pH in the production of mycotoxins by *Fusarium spp.* has been noted previously (Miller et al., 1983; Vidal et al., 1984).

Attempts to try and stimulate toxin production by increasing the amount of glucose in the medium had the reverse effect; i.e., a reduction in toxin production was observed when glucose levels were increased from 10 or 20 to 30 or 40 g/L. This is again in contrast to the situation with *F. moniliforme* where MYRO medium containing 40 g/L glucose supported the greatest production of fusarin C (Farber and Sanders, 1986) but is similar to the work of Miller et al. (1983) who found that increasing the glucose concentration of GYEP medium led to decreased mycotoxin yields.

The differences in the effects of pH, media, temperature, and carbohydrate levels on the production of fusarin C by *F. moniliforme* and *F. graminearum* in liquid culture suggest possibly that the organisms are producing fusarin C in response to different environmental stresses, i.e. nutrient limitations.

The amounts of fusarin C produced by various *Fusarium spp.* growing on corn is shown in Table III. Yields of fusarin C by Canadian isolates of *F. graminearum* were much higher than those reported by Gelderblom et al.

Table III. Amounts of Fusarin C Produced by Various *Fusarium spp.* Growing on Corn for 21 Days at 28 °C in an Atmosphere of 70% Relative Humidity

	fungus	fusarin C,[a] μg/g		fungus	fusarin C,[a] μg/g
F. graminearum			*F. poae*		
DAOM	180378	73.9	DAOM	177424	1.1
	178148	71.2	1133/28A		9.05
	180379	164.3	*F. culmorum*		
	178151	9.9		F2	9.5
	180376	30.0		F3	24.8
	180377	37.3		F7	9.3
	178150	61.9	ATCC	34913	73.3
	178149	42.2	*F. sambucinum*		
	177408	295.1	DAOM	170324	1.3
	177406	72.4	*F. avenaceum*		
F. sporotrichioides			ITFRC	R-4608	2.5
DAOM	175518	4.0	*F. acuminatum* (1)[b]		ND[c]
	177418	1.0	*F. equiseti* (2)		ND
	177420	1.1	*F. semitectum* (1)		ND
	180373	8.0	*F. solani* (3)		ND
1065/30B		6.9			
1136/17B		0.9			

[a] Average of duplicate determinations. [b] Number of strains tested. [c] ND not detected.

(1984b) for South African isolates. The significance of this is unclear at present. As shown previously (see Farber and Sanders (1986); Tables I–III, this paper) slight changes in aeration, temperature, and pH can have dramatic effects on biosynthesis of fusarin C by *Fusarium spp.* However, the effect of strain differences due to the geographical origin of the fungi cannot be ruled out.

It is quite interesting to note that most of the *F. graminearum* strains that produced fusarin C have also been shown capable of producing deoxynivalenol, acetyldeoxynivalenol, and zearalenone (Miller et al., 1983). Additionally, the *F. avenaceum* strain that produced fusarin C is a known moniliformin producer (Marasas et al., 1984b). Whether these compounds can interact synergistically in vivo to produce greater toxicological effects or whether they can be produced in combination in nature is unknown.

That fusarin C can be produced by as many as seven different species of *Fusarium* is not surprising. T-2 toxin, another important *Fusarium* mycotoxin, can be made by at least seven different *Fusarium spp.* (Marasas et al., 1984b). The significance, however, of the production of a highly mutagenic yet unstable compound by so many species of *Fusarium* remains to be answered.

This is the first report of fusarin C production by species of *Fusarium* other than *F. moniliforme* and *F. graminearum* as well as the first report on the ability of Canadian species of *Fusarium* other than *F. moniliforme* to produce this mycotoxin. Studies are continuing to determine whether fusarin C can appear as a natural contaminant in Canadian grains.

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Condensed Tannins: (4β→8;2β→*O*→7)-Linked Procyanidins in *Arachis hypogea* L.

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Mature, red peanut skins contain about 17% by weight of procyanidins. Nearly 50% of these compounds are low molecular weight oligomers that are soluble in ethyl acetate. The flavan-3-ols catechin and epicatechin are present in a ratio of 9 to 1 but in low concentration. Among the dimeric procyanidins, epicatechin-(4β→8;2β→*O*→7)-catechin dominates, with smaller proportions of epicatechin-(4β→8;-2β→*O*→7)-epicatechin and very small proportions of epicatechin-(4β→8)-catechin. Higher oligomers that are soluble in ethyl acetate contain both the (4β→8 or →6) and (4β→8;2β→*O*→7) interflavanoid bonds. The water-soluble polymeric procyanidins are predominately 2,3-*cis*-procyanidins linked by (4β→8 or →6) bonds that are terminated with the 2,3-*trans*-flavan-3-ol catechin. Water-soluble polymeric procyanidins from flaked and pelletized peanut skins have comparatively low number-average molecular weights (about 2200 as the peracetate) and low dispersitivities (about 1.8). These polymers have excellent potential for use in cold-setting adhesive applications.

INTRODUCTION

The skins of mature peanuts (*Arachis hypogea* L.) contain condensed tannins of the procyanidin class, but little is known of their structure (Stansbury et al., 1950; Sanders, 1977; Sanders and Mixon 1978). These tannins are credited with fungistatic properties inhibiting the development of *Aspergillus parasiticus* (Lansden, 1982; Sanders and Mixon, 1978). Approximately 40 000 dry tons of peanut skins is produced annually in the United States, and their high protein (about 17%) and fat (about 5%) contents have prompted their use as animal feeds. How-

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