represents an apomictic complex, *P. argentatum* is an outcrossing species with significant genetic diversity.

P. argentatum is the only species in the Parthenichaeta which contains bornyl acetate. We have found only one other species, in the herbaceous section, Argyrochaeta, *P. confertum* var. *lyratum*, which also contained bornyl acetate. This suggests the presence of a common biosynthetic pathway and infers a close or analogous genetic basis so that these two can serve as a bridge between the two sections, Parthenichaeta and Argyrochaeta.

P. hysterophorus is the most advanced herbaceous species. It is an aggressive weed which successfully spread northeast into Texas. It is highly allergenic (Towers et al., 1977; Loukar et al., 1974). One of us contracted an itchy rash on the hand which spread discontinuously to other places and was acute for about three weeks. Lingering residual effects persisted for over a year.

The absence of bornyl acetate is perhaps puzzling because it arose in *P. argentatum*, persisted in *P. confertum*, and then vanished in *P. hysterophorus*. From a presumed chromosome base number of $x = 6$, the woody species of the Parthenichaeta acquired $n = 18$, as well as *P.* confertum of Argyrochaeta (Rollins, 1950).

P. hysterophorus has lost one chromosome ($n = 17$) and this lends credence to the plausibility that the absence of bornyl acetate may be associated with the missing chromosome.

Abreviations used: FW, fresh weight.

Registry No. Methanol, 67-56-1; ethanol, 64-17-5; α-pinene, 80-56-8; thujene, 58037-87-9; camphene, 79-92-5; β-pinene, 127-91-3; sabinene, 3387-41-5; β-myrcene, 123-35-3; α-terpinene, 99-86-5; limonene, 138-86-3; terpinolene, 586-62-9; 1,8-cineole, 470-82-6; β-ocimene, 13877-91-3; α-ocimene, 502-99-8; *p*-cymene, 535-77-3; β-phellandrene, 555-10-2; hexanol, 111-27-3; *cis*-3-hexenol, 928-96-1; linalool, 78-70-6; bornyl acetate, 76-49-3; caryophyllene, 87-44-5; humulene, 6753-98-6; terpinen-4-ol, 562-74-3.

LITERATURE CITED

Alexander, P. *Ber. Dtsch. Chem. Ges.* **1911**, *44*, 2320.
Calvin, M. *Photochem. Photobiol.* **1976**, *23*, 425.
Dominguez, X. A.; Gomez, E.; Gomez, P. A.; Vilarreal, A. N.; Rombold, C. *Planta Med.* **1971**, *19*, 52.
Haagen-Smit, A. J.; Siu, R. *J. Am. Chem. Soc.* **1944**, *66*, 2068.
Kumamoto, J.; Scora, R. W. *J. Agric. Food Chem.* **1984**, *32*, 418.
Loukar, A.; Mitchell, J. C.; Calnan, C. D. *Trans. St. John's Hosp. Dermatol. Soc.* **1974**, *60*, 43.
Rodriguez, E. "The chemistry and distribution of sesquiterpene lactones and flavonoids in *Parthenium* (Compositae): Systematic and ecological implications"; Xerox University Microfilms: Ann Arbor, MI, 1975; pp 308.
Rollins, R. C. *Contrib. Gray Herb. Harv. Univ.* **1950**, *172*, 1.
Scora, R. W.; Kumamoto, J. *J. Agric. Food Chem.* **1979**, *27*, 642.
Towers, G. H.; Mitchell, J. G.; Rodriguez, E.; Bennett, F. D.; Subba Rao, P. V. *J. Sci. Ind. Res.* **1977**, *36*, 672.

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Fusarin C: Structure Determination by Natural Abundance ^{13}C–^{13}C Coupling and Deuterium-Induced ^{13}C Shifts

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The structure of fusarin C, a relatively potent mutagen produced by the common fungus *Fusarium moniliforme*, was determined principally by analysis of NMR data. The carbon backbone was established by analysis of natural abundance ^{13}C–^{13}C coupling patterns and positions of oxygen and nitrogen substitution were established by analysis of deuterium-induced shifts in resonances of certain carbon atoms. Stereochemistries of double bonds were established by analysis of vicinal ^{13}C–^{1}H and vicinal ^{1}H–^{1}H couplings.

Fusarin C is the major bacterial mutagen produced by *Fusarium moniliforme* grown on corn under laboratory and field conditions (Wiebe and Bjeldanes, 1981; Gelderblom et al., 1983; Gaddamidi and Bjeldanes, 1984). Corn infected with *F. moniliforme* caused cancer in laboratory animals (Yang, 1980) and has been associated with cancer in human populations in Africa and China (Marasas et al., 1981; Li et al., 1980). The structure elucidation of fusarin C depended primarily on long-range ^{1}H–^{13}C and natural abundance ^{13}C–^{13}C coupling as determined from a two-dimensional double-quantum coherence experiment (Mareci and Freeman, 1982; Bax et al., 1981). Analyses of deuterium-induced shifts in appropriate ^{13}C resonances provided a basis for assignments of oxygen and nitrogen substitution (Pfeffer et al., 1979).

Fusarin C (1) is a yellow oil $[\alpha]^{23}$ +47.04 (2.0% in MeOH) isolated from *F. moniliforme* grown on cracked corn (Wiebe and Bjeldanes, 1981). The mutagenic fraction was passed sequentially through columns of LH-20 (MeOH), silica gel (MeOH–CH_2Cl_2), and LH-20 (MeOH). Purification was completed by semipreparative chromatography on a low-pressure silica gel column (Si60, size B, 2.0% MeOH in CH_2Cl_2). The molecular formula, $C_{23}H_{29}NO_7$, was established by combustion analysis and by high-resolution mass spectral analysis (m/e found 431.1953, required 431.1993). The UV absorptions at 365 nm (ε 25 655) and 265 nm (ε 8250) are characteristic of a substituted polyene. The IR spectrum showed intense absorption bands for OH and NH at 3300–3600 cm^{-1} and for γ-lactam, ester, and conjugated ketone carbonyls at

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Table I. Proton and Carbon-13 Data for Fusarin C

C	H[a] at C [multiplicity, J (Hz)]	C	J_{C-H}	$^{13}C-^{13}C$ coupled pairs	$^3J_{H-C-C-C}$[b]
1		170.36 (s)		1–2	0.5 (H–3)
2		61.93 (s)		2–1′, 2–3, 2–1	
3	4.02 (1 H, d, 2.7)	63.75 (d)	175	3–2, 3–4	
4		85.43 (s)		4–3, 4–18′	
1′		190.36 (s)		1′–2′, 1′–2	6.8 (H–3′), 1.5 (H–3)
2′		133.38 (s)		2′–1′, 2′–13′, 2′–3′	
3′	7.52 (1 H, d, 12.0)	146.41 (d)	128	3′–2′, 3′–4′	
4′	6.61 (1 H, dd, 12.0 and 15)	123.39 (d)	134	4′–3′, 4′–5′	
5′	6.80 (1 H, d, 15.0)	149.37 (d)	132	5′–4′, 5′–6′	
6′		134.85 (s)		6′–5′, 6′–7′, 6′–14′	
7′	6.29 (1 H, s)	140.95 (d)	151	7′–6′, 7′–8′	
8′		137.41 (s)		8′–7′, 8′–9′, 8′–15′	
9′	6.08 (1 H, s)	126.20 (d)	134	9′–8′, 9′–10′	
10′		130.29 (s)		10′–9′, 10′–11′	
11′	6.99 (1 H, q, 7.2)	140.23 (d)	155	11′–10′, 11′–12′	
12′	1.79 (3 H, d, 7.2)	16.04 (q)	110	12′–11′	
13′	2.09 (3 H, s)	11.45 (q)	110	13′–2′	7.5 (H–3′)
14′	2.0 (3 H, s)	14.09 (q)	110	14′–6′	5.0 (H–5′), 8.0 (H–7′)
15′	1.72 (3 H, s)	18.79 (q)	110	15′–8′	4.0 (H–7′), 9.0 (H–9′)
16′		167.65 (s)			6.8 (H–11′), 1.0 (H–17′)
17′	3.75 (3 H, s)	51.95 (q)	125	10′–17′ (C–O–C)	
18′	2.08 (2 H, m)	35.97 (t)	135	18′–4, 18′–19	
19′	4.0 (2 H, m)	58.05 (t)	127	19′–18′	

[a] 200 MHz, $CDCl_3$. [b] 62.5 MHz, $CDCl_3$. [c] 75 MHz, $CDCl_3$.

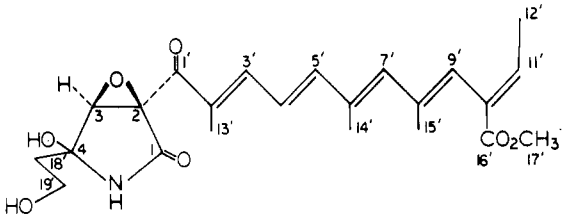

Figure 1. Structure of fusarin C.

1735, 1725, and 1665 cm^{-1}, respectively.

The NMR spectral data are summarized in Table I. Proton–proton decoupling experiments confirmed that the proton pairs H-12′ and H-11′ and H-18′ and H-19′ were on adjacent carbons and that the vinyl protons at H-3′, H-4′, and H-5′ were on contiguous carbons. The 4′*E* stereochemistry is established by the 15-Hz coupling observed for H-4′ and H-5′. A further decoupling experiment established that the weak coupling (J = 2.7 Hz) of H-3 was due to the amide proton. The ^{13}C NMR spectrum (62.5 MHz, $CDCl_3$) showed resonances for carbonyl carbons of a conjugated ketone (C-1′, δ 190.36), a conjugated ester (C-16′, δ 167.65), and a lactam (C-1, δ 170.36). Signals were present for ten vinyl carbons (C-2′ through C-11′, δ 123.39–146.41), four carbons singly substituted with heteroatoms (C-2, δ 61.93, C-3, δ 63.75, C-19′, δ 58.05, C-17′, δ 51.95), a carbon substituted with two heteroatoms (C-4, δ 85.43), a dialkyl-substituted carbon (C-18′, δ 35.97), and four methyl groups (C-12′ through C-15′, δ 11.45–18.79).

To establish the carbon backbone for fusarin C the natural abundance $^{13}C-^{13}C$ one-bond couplings were determined by using a 2-D INADEQUATE experiment. The observed $^{13}C-^{13}C$ couplings are indicated in Table I.

A long-range heteronuclear correlation experiment optimized for 5 Hz indicated (Table I) a three-bond coupling between C-16′ and H-11′ thus establishing the position of the carbomethoxyl unit at C-10′. The *E* stereochemistry of the 2′–3′ double bond was established by comparison of $^3J_{COR,H}$ (6.8 Hz, cis) and $^3J_{CH_3,H}$ (7.5 Hz, trans). For the similarly substituted 10′–11′ double bond the *E* stereochemistry was established by an identical $^3J_{COOCH_3,H}$ (6.8 Hz). The vicinal couplings for the 6′–7′ (8 Hz) and 8′–9′ (9 Hz) double bonds are in accord with the *E* stereochemical assignments in light of established steric effects of substituents on olefins (Marshall, 1983).

The aforegoing information established the carbon skelton, γ-lactam functionality, and double bond stereochemistry for fusarin C indicated in Figure 1.

Sites of oxygen substitution were established by results by acylation experiments (0.27 ppm downfield shift of H-19′ signals in the *p*-nitrobenzoate of fusarin C) and deuterium-induced shifts in certain ^{13}C NMR signals. The spectrum of a D_2O-exchanged sample of fusarin C showed that the signal assigned to C-4 (δ 85.43 in the undeuterated sample) appeared as four signals separated by approximately 0.1 ppm. These signals indicated that the two heteroatoms to which C-4 is bonded are attached to two exchangeable protons and establish the presence of a hydroxyl group at C-4. Splits of similar magnitude (two signals, Δ δ 0.1) were noted for the C-1 and C-19′ signals, confirming that they are adjacent to NH and OH groups, respectively. Only line broadening (γ-effect) was observed for signals of C-3 and C-18′. No broadening was observed for the C-2 signal. The results clearly establish the structure of fusarin C as indicated in Figure 1. The cis stereochemistry of the epoxide is assumed on the basis of ring strain considerations. To our knowledge the cyclic aminol epoxide moiety of fusarin C has precedent in natural products only in cerulenin, a biologically active metabolite of *Cephalosporium caerulens* (Boeckman, Jr. and Thomas, 1979; Jakubowski et al., 1982). The numbering system indicated in Figure 1 is based on the numbering systems accepted for cerulenin (Funabashi et al., 1983) and other natural products (Wat et al., 1977). The proposed structure of fusarin C is in accord with the structure proposed recently based on an X-ray diffraction study of a photoproduct (Gelderblom et al., 1984).

Registry No. Fusarin C, 79748-81-5.

LITERATURE CITED

Bax, A.; Freeman, R.; Frenkiel, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 2102.

Boeckman, Jr., R. K.; Thomas, E. W. *J. Am. Chem. Soc.* **1979**, *101*, 987.

Funabashi, H.; Iwasaki, S.; Okuda, S. *Tetrahedron Lett.* **1983**, *24*, 2673.

Gaddamidi, V; Bjeldanes, L. F., unpublished findings.

Gelderblom, W. C. A.; Marasas, W. F. O.; Steyn, P. S.; Thiel, P. G.; van der Merwe, K. J. (in part); van Rooyen, P. H.; Vleggaar,

R.; Wessels, P. L. *J. Chem. Soc., Chem. Commun.* **1984**, 122.
Gelderblom, W. C. A.; Thiel, P. G.; van der Merwe, K. J.; Marasas, W. F. O.; Spies, H. S. *Toxicon* **1983**, *21*, 467.
Jakubowski, A. A.; Guziec, Jr., F. S.; Sugiura, M.; Tam, C. C.; Tishler, M.; Omura, S. *J. Org. Chem.* **1982**, *47*, 1221.
Li, M.-H.; Lu, S.-H.; Ji, C.; Wang, Y.; Wang, M.; Cheng, S.; Tian, G. "Genetic and Environmental Factors in Experimental and Human Cancer"; Gelboin, H. V.; MacMahon, B.; Matsushima, T.; Sugimura, T.; Takayama, S.; Takebe, H., Eds.; Japanese Science Society Press: Tokyo, 1980; pp 139–148.
Marasas, W. F. O.; Wehner, F. C.; vanRensberg, S. J.; van-Schalkwyk, D. J. *Phytopathology* **1981**, *71*, 792.
Mareci, T. H.; Freeman, R. *J. Magn. Reson.* **1982**, 293.
Marshall, J. L. "Methods in Stereochemical Analysis, 2: Carbon-Carbon and Carbon-Proton NMR Couplings: Applications to Oranic Stereochemistry and Conformational Analysis"; Marchand, A. P., Ed.; Verlag Chemie International Inc.: Deerfield Beach, FA, 1983; pp 33–38.
Pfeffer, P. E.; Valentine, K. M.; Parrish, F. W. *J. Am. Chem. Soc.* **1979**, *101*, 1265.
Wat, C.-K.; McInnes, A. G.; Smith, D. G.; Wright, J. L. C.; Vining, L. C. *Can. J. Chem.* **1977**, *55*, 4090.
Wiebe, L. A.; Bjeldanes, L. F. *J. Food Sci.* **1981**, *46*, 1424.
Yang, C. S. *Cancer Res.* **1980**, *40*, 2633.

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Surface Wax of Coastal Bermuda Grass

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The surface cuticular wax of Coastal Bermuda grass was separated into hydrocarbons (10%), esters (33%), free alcohols (28%), free aldehydes (3%), and free acids (12%) by column chromatography. The composition of the individual fractions was determined by gas liquid chromatography (GLC) by comparison with known compounds. The major hydrocarbon was identified as tritriacontane. The ester fraction was hydrolyzed and the principle alcohols were identified as docosanol, tetracosanol, hexacosanol, and octacosanol. The major acids contained in the esters were identified as eicosanoic and docosanoic acids. The principle free constituents were found to be alcohol (unknown triterpenol), aldehyde (hexadecanal), and acid (hexadecanoic). The presence of tritriacontane (C33) as the major hydrocarbon is unusual as it is rarely a major component of plant waxes.

INTRODUCTION

The significance of plant waxes in the control of important biological phenomena as water loss, agricultural spray efficiency, and mechanical leaf damage has been well established (Eglinton and Hamilton, 1967). However, the literature contains very few reports on the analysis of surface waxes of forage plants. Ryegrass leaf wax (*Lolium perenne*) has been investigated by several workers (Pollard et al., 1931; Hamilton and Power, 1969; Allebone et al., 1970; Allebone and Hamilton, 1972). The surface wax of sorghum (*Sorghum bicolor*) (Bianchi et al., 1978; Wilkinson and Cummins, 1981) and corn (*Zea mays*) (Bianchi et al., 1975) has been studied. The surface wax composition has also been determined on wheat varieties by Tulloch and co-workers (Tulloch and Weenink, 1969; Tulloch and Hoffman, 1973), millets (Tulloch, 1982), and on sweet clover (*Metilotus alba*) by Emery and Gear (1969). There have been no reports on the composition of the surface wax of warm season grasses. Coastal Bermuda grass (*Cynodon dactylon*) is an important warm season forage crop in the southern United States that is dehydrated, grazed, hayed, and prepared as silage for use in animal feeds. In our continued investigation of the physical, chemical, and structural differences between cool and warm season grasses, the composition of the surface wax of Coastal Bermuda grass has been determined.

MATERIALS AND METHODS

Extraction of Surface Wax. Coastal Bermuda grass [*Cynodon dactylon* (L.) Pers] was harvested after 24–30 days regrowth. The grass was fertilized with 380/112/112 Kg (N/P/K) per hectare in split applications during the growing season. The fresh grass was dipped twice in 1 liter of hexane for 20 s at room temperature. Fresh solvent was used for each dipping. Care was taken not to get the cut ends into the hexane solution. The hexane extracts were combined and filtered, and the solvent removed under vacuum to yield the crude wax as a light yellow solid. Infrared spectra were run on a Beckman IR-8 in Nujol or in carbon tetrachloride solution. Silica gel 60 was used for thin-layer chromatography and developed with chloroform containing 1% ethanol (Tulloch and Hoffman, 1971).

Separation of Wax Components. The extracted wax components were separated by column chromatography by using stepwise elution as described by Tulloch and Weenink (1969). Crude wax was applied to an unconditioned silica gel 60 column (2 × 18 cm) and 50 (100 mL) fractions were collected. The hydrocarbons were eluted in fractions 1–7 with hexane. Hexane–chloroform (75:25, v/v) eluted the esters in fractions 8–15. The same solvents 25:75, v/v) eluted the alcohols in fractions 20–26 followed by the aldehydes in fractions 27–34. Free acids were eluted with chloroform in fractions 39–42 and unidentified materials were eluted by methanol in the remaining fractions. The recovery was 97% of the crude wax applied to the column.

Fresh Coastal Bermuda grass was extracted in 1-kg batches. A total of 3–4 kg was extracted in this manner. Typical yields of surface wax were 0.8–1.0% on a dry weight basis. Infrared spectra of the crude wax gave a broad carbonyl absorption at 1710–1730 cm^{-1} and a broad hydroxyl absorption of 3500–3600 cm^{-1}. Thin-layer chromatography showed the presence of hydrocarbons, esters, alcohols, and free acids in addition to some unknown material in the crude wax. GLC system B was also used

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