Viridoxins A and B: Novel Toxins from the Fungus *Metarhizium flavoviride*

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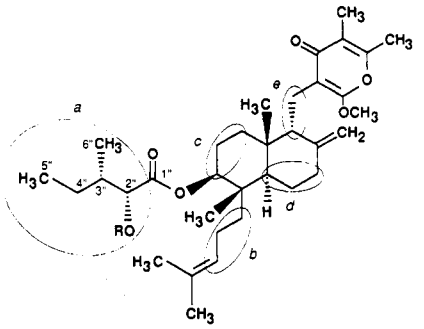
The structures of viridoxins A and B, two novel toxins isolated from the mycelial extract of the fungus *Metarhizium flavoviride*, were established by spectroscopic methods, chemical correlations, and a single-crystal X-ray analysis of viridoxin B. The absolute configuration of the viridoxins was determined by ^{1}H NMR analysis of chiral (*R*)- and (*S*)-*O*-methyl mandelate derivatives of viridoxin A.

Entomopathogenic fungi are known to produce diverse secondary metabolites, some of which have toxic properties.[1] Several species of *Metarhizium* (Deuteromycotina, Hyphomycetes) are known to be pathogenic to a wide range of insects,[2] particularly soil-inhabiting insects, and production of toxic metabolites by these fungi is believed to play a significant role in the pathogenicity and host survival. In this paper we report the isolation and structure elucidation of two novel metabolites—viridoxins A and B from the fungus *Metarhizium flavoviride*. Both metabolites are fairly potent insect toxins. There are no previous reports of isolation of secondary metabolites from *M. flavoviride*. Another species of *Metarhizium*, *M. anisopliae*, produces toxic cyclodepsipeptides, the destruxins.[3]

M. flavoviride was cultured in Sabouraud Dextrose medium and the mycelium separated by filtration. The methylene chloride soluble fraction from an ethanolic extract of the mycelium afforded viridoxin A and B upon bioassay-guided separation by silica gel chromatography followed by reversed-phase HPLC. Viridoxin A (1) was



1 R = H
2 R = COCH$_3$

isolated as a white crystalline solid. The molecular formula $C_{34}H_{52}O_6$, as determined by high-resolution FAB mass spectrometry, suggested the presence of nine degrees of unsaturation. The ^{13}C NMR spectrum confirmed the presence of 34 carbons, of which nine were methyls, nine were methylenes, and six were methines as determined by a DEPT analysis.[4] Acetylation furnished a monoacetylated derivative (2) ($M + H^+$ m/z 599, FAB mass spectrum) suggesting the presence of a single hydroxyl function. That this was a secondary hydroxyl was confirmed by the downfield shift of a single proton in ^{1}H NMR (signal at 4.23 ppm in 1 is shifted to 5.36 ppm in 2).[5] The acetylation also resulted in the disappearance of the hydroxyl proton resonance at 2.96 ppm.

Analysis of the ^{1}H NMR spectral data using a COSYPS[6] experiment revealed the presence of several (*a*–*e*) isolated spin systems in 1. From the information derived from the DEPT analysis combined with the ^{13}C-^{1}H HETCOR[7] experiment, all the signals in the ^{1}H NMR spectrum could be assigned to individual methine, methylene, and methyls. The spin system *a* with α-hydroxy methine at 4.23 ppm was a part of an α-hydroxy acid moiety. This was confirmed by the presence of a long-range connectivity in a COLOC[8] experiment between the carbonyl carbon at 174.93 ppm and the 2″ proton at 4.23 ppm and the hydroxy proton at 2.96 ppm. In the spin system *b*, the methine at 5.19 ppm was placed next to a double bond because of the chemical shift consideration and observation of long-range COLOC connectivity between C-15 and C-16 methyl carbons and the C-13 methine proton, and the C-11 methylene carbon and the C-17 methyl protons. In the spin system *c*, the methine at 5.18 ppm showed a long-range connectivity to the C-17 methyl protons in COSY-

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(2) Roberts, D. W.; Humber, R. A. In *Biology of Conidial Fungi*; Cole, G. T.; Kendrick, B., Eds.; Academic Press: New York, 1981; Vol. 2, p 201.

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(6) Benn, R.; Gunther, H. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 350. Bax, A. In *Two Dimensional Nuclear Magnetic Resonance In Liquids*; Reidel: Boston, 1982.

(7) Bax, A.; Morris, G. A. *J. Magn. Reson.* **1981**, *42*, 501. Freeman, R. *Proc. R. Soc. London A* **1980**, *373*, 149. Rutar, V. *J. Am. Chem. Soc.* **1983**, *105*, 4095.

(8) Kessler, H.; Griesinger, C.; Zarbock, J.; Loosli, H. R. *J. Magn. Reson.* **1984**, *57*, 331. Krishnamurthy, V. V.; Casida, J. E. *Magn. Reson. Chem.* **1987**, *25*, 837. COLOC of 1 was recorded with Varian-supplied pulse sequence (COLOCS = n, COUPLD = n). The experiment was performed using fixed evolution time d3 of 60 ms and a refocusing delay d4 of 32 ms.

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Table I. ^{1}H NMR Spectral Data for 1, 3, and 5[a]

H no.	chemical shifts (ppm) in benzene-d_6 (multiplicity, J in Hz) 1	3	5
1	1.34, 1.94 (each m)	1.35, 1.95 (each m)	1.44, 1.99 (each m)*
2	1.7, 1.77 (each m)	1.7, 1.75 (each m)	1.64 (m)*
3	5.18 (m)	5.18 (m)	3.5 (m)
5	1.91 (m)	1.92 (m)	1.85 (m)*
6	1.37, 1.53 (each m)	1.38, 1.53 (each m)	1.44, 1.61 (each m)*
7	2.14, 2.62 (each m)	2.14, 2.63 (each m)	2.17, 2.63 (each m)*
9	2.36 (m)	2.37 (m)	2.34 (m)
11	1.36 (m)	1.37 (m)	1.41 (m)*
12	2.09, 2.26 (each m)	2.06, 2.28 (each m)	2.17 (m)*
13	5.19 (m)	5.2 (m)	5.28 (m)
15	1.67 (s)+	1.68 (s)+	1.67 (s)+
16	1.69 (s)+	1.69 (s)+	1.72 (s)+
17	0.88 (s)	0.88 (s)	0.89 (s)
18	4.52. 4.68 (each m)	4.53, 4.69 (each m)	4.5, 4.68 (each m)
19	2.72, 2.85 (each m)	2.73, 2.86 (each m)	2.77, 3.03 (each m)
20	1.0 (s)	1.0 (s)	1.06 (s)
7′	3.27 (s)	3.26 (s)	3.24 (s)
8′	1.86 (s)	1.86 (s)	1.85 (s)
9′	1.5 (s)	1.49 (s)	1.47 (s)
2″	4.23 (m)	4.24 (m)	–
OH	2.96 (m)	2.83 (m)	–
3″	1.86 (m)	1.62 (m)	–
4″	1.36, 1.63 (each m)	2.01 (m)	–
5″	0.9 (t, 7.4)	0.92 (d, 6.8)#	–
6″	0.93 (d, 6.8)	0.93 (d, 6.6)#	–

[a] Assignments marked with an * are tentative and are based on comparison with the data from 1. +, # assignments may be reversed within the column.

45, and long-range COLOC connectivities were observed for the quaternary carbons C-4 and C-10 with the C-17 methyl and C-20 methyl protons respectively (Tables I and II).

The methine proton (C-5) at 1.91 ppm, part of the spin system *d*, a $-CH_2CH_2CH-$ system, showed a long-range connectivity in COSY-45 to the methyl protons at C-20 (1.0 ppm), and a long-range connectivity was observed between the C-6 methylene protons at 1.35 and 1.53 ppm and the C-18 methylene protons. The placement of this spin system, as in 1, was further substantiated by the observation of several long-range COLOC connectivities between the C-5,C-6, and C-7 carbon resonances to the C-17, C-5, and C-18 proton resonances, respectively. The last spin system (*e*), derivable from the COSYPS connectivities, was the $-CH_2CH-$ system where C-19 methylene resonances at 2.72 and 2.85 ppm showed a long-range connectivity to the methoxy protons at 3.27 ppm in COSY-45. The assignment of the spin systems as in 1, and the placement of quaternary carbons C-14, C-4, C-8, and C-10, was further substantiated by long-range COLOC connectivities as shown in Table II. On the basis of the above information the partial structure could be constructed except for a fragment which would account for one carbonyl (^{13}C signal at 180.26 ppm), one oxygen, one methoxyl (3.27 ppm), four quaternary carbons, and two methyls bound to double bonds (^{1}H signals at 1.5 and 1.86 ppm). A UV absorption at 258 nm for 1 suggested the presence of a γ-pyrone moiety which was further supported by the observation of several long-range connectivities in the COLOC experiments—C-3′ carbon and C-19 methylene protons; C-4′ carbon (carbonyl) and C-19 methylene protons and C-8′ methyl protons; C-5′ carbon and C-8′ methyl protons and C-9′ methyl protons; C-6′ carbon and C-8′ methyl protons and C-9′ methyl protons; and C-2′ carbon and C-7′ methoxyl protons and C-19 methylene protons. The combined evidence led to the structure assignment as in 1. The complete ^{1}H and ^{13}C NMR spectral assignments for 1 as derived from a combination

Table II. ^{13}C NMR Spectral Data for 1, 3, and 5 and COLOC Connectivities for 1[a]

C no.	chemical shifts (ppm) in benzene-d_6 1	COLOC correlation to H no.	3	5
1	33.65	H-20	34.2	34.78
2	24.05	H-20, H-17	24.46	28.42
3	78.04	H-17, H-1	77.82	73.68
4	40.06	H-5, H-17, H-2	40.45	41.36
5	39.12	H-7, H-17, H-20	39.48	39.4
6	22.62	H-5	23.1	23.4
7	30.61	H-18	31.28	31.49
8	148.43	H-7, H-6, H-9, H-19	149.34	149.8
9	55.45	H-20, H-18	55.96	56.42
10	37.37	H-20, H-2, H-1, H-6	37.85	38.16
11	37.74	H-17	38.26	38.04
12	21.7		22.28	22.45
13	124.34	H-15	125.15	126.04
14	131.47	H-15	131.19	130.53
15	17.52*	H-13	17.62*	17.4*
16	25.72*	H-13	25.9*	25.93*
17	18.13	H-2, H-3, H-5	18.36	17.71
18	109.59	H-7, H-9	109.71	109.42
19	19.91	H-9	20.63	20.55
20	22.87	H-1, H-2, H-5	23.38	23.46
2′	162.81	H-7′, H-19	162.44	162.6
3′	103.07	H-19	103.44	103.67
4′	180.26	H-19, H-8′	179.41	179.53
5′	118.57	H-8′, H-9′	118.91	118.89
6′	154.89	H-8′, H-9′	154.35	154.38
7′	55.31		54.77	54.75
8′	10.0		10.2	10.22
9′	16.96		16.1	16.1
1″	174.93	OH, H-2″	175.67	
2″	72.98	OH, H-6″	69.24	
3″	38.59	H-6″, H-5″	44.02	
4″	25.8	H-5″	24.86	
5″	11.89		23.27+	
6″	13.14	H-2″	21.9+	

[a] All the carbon multiplicities were confirmed by DEPT analysis. *, + assignments may be reversed within the column.

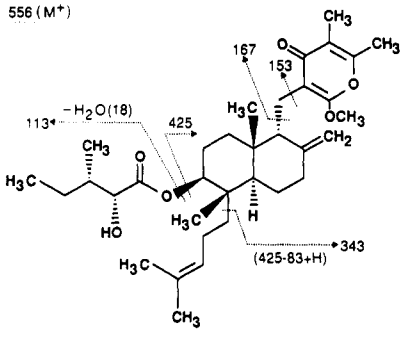


Figure 1. Mass spectral fragmentation pattern for 1.

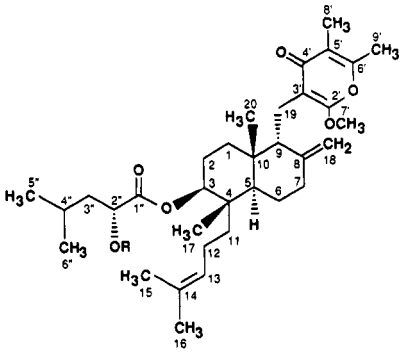
of COSYPS, COSY-45, ^{1}H HOM2DJ resolved,[9] DEPT, HETCOR, and COLOC experiments are given in Tables I and II, respectively.

The EI mass spectrum of 1, in addition to the molecular ion, showed several characteristic fragment ions. The fragmentation pattern for 1 is shown in Figure 1. The EI mass spectrum of 1 showed a molecular ion at *m/z* 556 along with characteristic fragment ions such as at *m/z* 425 (cleavage between C-3 and oxygen), *m/z* 343 (loss of aliphatic side chain concomitant with a hydrogen transfer from the fragment at *m/z* 425), *m/z* 167 (the most intense peak above *m/z* 100, generated by cleavage of C-9–C-19 bond with the charge retention on γ-pyrone moiety), *m/z* 153 (cleavage of C-19–C-3′ bond), and *m/z* 113 (cleavage

(9) Wernly, L.; Lauterwein, J. *Helv. Chim. Acta* **1983**, *66*, 1576. Freeman, R.; Morris, G. A. *Bull. Magn. Reson.* **1979**, *1*, 5.

of C-3–O bond along with the loss of a water molecule from the α-hydroxy acid moiety). The HRFAB mass spectrum of 1 showed an intense protonated molecular ion at m/z 557.3834 ($C_{34}H_{53}O_6$, calcualted 557.3842, Δ mmu 0.8).

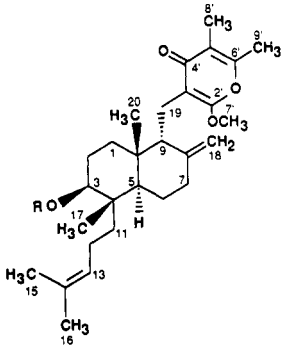
The minor toxin, viridoxin B (3), was isolated as a crystalline white solid. The molecular formula was determined to be $C_{34}H_{52}O_6$ (M + H$^+$ 557.3853, calculated 557.3842, Δ mmu 1.1) identical to that of 1 by HRFABMS.



3 R = H
4 R = COCH$_3$

The ^{1}H NMR of 3 (Table I) was similar to that of 1, except for the replacement of the methyl triplet at 0.93 ppm in 1 with a doublet at 0.93 ppm in 3. This observation in conjunction with the identical molecular formulas for 1 and 3 could be explained by placing the C-6″ methyl at C-4″ in 3 as opposed to its placement at C-3″ in 1. The ^{13}C NMR of 3 (Table II) showed 34 carbons which were resolved into nine methyls, nine methylenes, and six methines by DEPT analysis. Acetylation of 3 furnished a monoacetylated product (4) which showed a protonated molecular ion at m/z 599 in the FAB mass spectrum.

The assigned structures of 1 and 3 were confirmed by their base-catalyzed hydrolyses to furnish an identical alcohol (5) in both cases. Alcohol 5 showed a protonated



5 R = H
6 R = COCH$_3$

molecular ion at m/z 443 in the FAB mass spectrum. The ^{1}H NMR showed the presence of seven methyls (four bonded to the double bonds C-8′, C-9′, C-15, and C-16, two singlets C-17 and C-20, and one methoxyl C-7′) and an olefinic methylene (C-18). The other characteristic resonances were for one α-hydroxy methine (C-3), an olefinic proton (C-13), and the downfield methylene (C-19). The UV spectrum of 5 showed an absorption maximum at 257 nm thus confirming the presence of a γ-pyrone moiety. The ^{13}C NMR of 5 showed the presence of 28 carbons. The tentative ^{1}H and ^{13}C NMR assignments

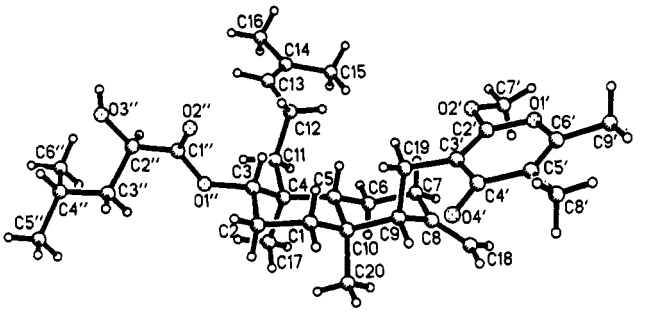
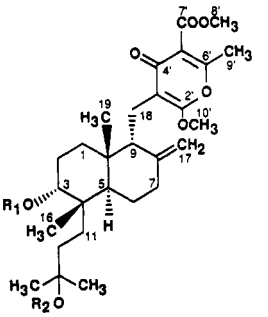


Figure 2. A computer-generated perspective drawing of 3.

for 5 are listed in Tables I and II, respectively. A literature search revealed that 5 was previously isolated as colletochin, a phytotoxin, from the plant pathogenic fungus *Colletotrichum nicotianae*.[10] Reported NMR data for colletochin were in close agreement with the data from 5. Acetylation of 5 furnished a monoacetylated derivative (6) which showed a protonated molecular ion at m/z 485 in the FAB mass spectrum. In addition to colletochin (5), *C. nicotianae* also produces structurally related phytotoxins colletotrichin (7) (also produced by *C. capsici*), colletotrichin B (8), and colletotrichin C (9) which are, like viridoxins 1 and 3, unique (nor) diterpene derivatives of polysubstituted γ-pyrones.[11]



7 R$_1$ = H, R$_2$ = H
8 R$_1$ = CHO, R$_2$ = H
9 R$_1$ = H, R$_2$ = CHO

An unambiguous confirmation for the assigned structures was obtained by a single-crystal X-ray analysis of viridoxin B (3). A computer-generated perspective drawing of compound 3 is shown in Figure 2. The fused six-membered rings assume a *trans*-decalin conformation. The γ-pyrone moiety is projected into an axial position with respect to the exocyclic unsaturation at C-8 and trans with respect to the methyl at C-10. The α-hydroxy-4-methylpentanoic acid assumes the equatorial position at C-3 and is disposed in a trans orientation with respect to the extended 2-ene-2-methylpentenoyl side chain at C-4. The molecules pack in an overall extended conformation, with an intermolecular hydrogen bond interaction between O-3″ and O-4′ (2.86 Å). All bond distances and angles are within expected values. Interestingly, the relative stereochemistry at C-3 of the viridoxins does not correspond to the C-3 stereochemistry of colletotrichin (7), which was structurally characterized by a single-crystal X-ray analysis

(10) Kimura, Y.; Hamasaki, T.; Suzuki, A. *Koen Yoshishu-Koryo, Terupen oyobi Seiyu Kagaku ni kansuru Toronaki*, 23rd, 1979, 288, Chem. Soc. Japan, Tokyo, Japan; *Chem. Abstr.* 1980, *93*, 65502e.

(11) Grove, J. F.; Speake, R. N.; Ward, G. *J. Chem. Soc. C* 1966, 230. Suzuki, A.; Gohbara, M.; Kosuge, Y.; Tamura, S.; Ohashi, Y.; Sasada, Y. *Agric. Biol. Chem.* 1976, *40*, 2505. Gohbara, M.; Kosuge, Y.; Yamasaki, S.; Kimura, Y.; Suzuki, A.; Tamura, S. *Agric. Biol. Chem.* 1978, *42*, 1037.

of 7 and its acetate.[12] The absolute stereochemistries of colletochin (5) and colletotrichins (7–9) from *C. nicotianae* have not been reported.

The absolute configuration of the viridoxins was determined by preparing chiral esters of 1 with (*R*)- and (*S*)-*O*-methylmandelic acids (10 and 11, respectively). It has been shown that esters of secondary alcohols with *O*-methylmandelic acid have a conformational preference where the methoxyl group of the mandelate eclipses the carbonyl group of the ester.[13] The absolute configuration is determinated by ^{1}H NMR analysis where only the substituent which is eclipsed by the phenyl ring shows an upfield shift of the resonances because of the aromatic shielding effect. Derivatization of 1 was accomplished by the DCC/pyridine/2-hydroxybenzotriazole method as reported by Trost et al.[13] 10 and 11 both showed protonated molecular ions at *m/z* 705 in their FAB mass spectra. Although some racemization was observed (~30% in the case of (*S*)-*O*-methyl mandelate and ~15% in the case of (*R*)-*O*-methyl mandelate), the ^{1}H NMR analysis of the resultant diastereomers clearly led to the assignment of the absolute configuration as *R* at the chiral center C-3″ in 1. The diastereomers produced as a result of the racemization did not separate under the chromatographic conditions used for their purification. As shown in Figure 3, the (*S*)-*O*-methyl mandelate derivative 11 showed C-5″ and C-6″ methyl resonances at 0.63 ppm (+0.09 ppm) and 0.96 ppm (+0.05), respectively, and C-17 and C-20 methyls at 0.94 and 1.02 ppm, respectively. The (*R*)-*O*-methyl mandelate (10) showed aromatic shielding of the methyls C-17 and C-20 which resonated at 0.85 ppm (+0.09 ppm) and 1.0 ppm (+0.02 ppm), respectively (see extended Newman projections for 10 and 11 in Figure 3). On the other hand, in 10 the proton resonances from C-5″ and C-6″ methyls were at 0.72 and 1.01 ppm, respectively. These results combined with the X-ray structure of 3 led to the assignment of the absolute stereochemistry of all the chiral centers in 3 as shown. The absolute stereochemistry of 1 was also determined to be as for 3 except for the chirality at C-3″ which was determined to be *S* by a 2D ^{1}H NOESY[14] experiment as follows.

1 was subjected to a NOESY analysis in benzene-d_6 with a mixing time of 200 ms. The NOESY connectivities as observed are shown in Figure 4. Of particular interest were the interactions between the hydroxyl proton and the C-6″ methyl and between the C-2″ methine and C-4″ methylene protons. These NOE interactions in conjunction with the previously established *R* configuration at the C-2″ led to the assignment of the *S* configuration at C-3″. A Newman projection through the C-3″–C-2″ bond emphasizing the relative spatial arrangement of the substituents around this bond is shown in Figure 5. The rest of the NOESY interactions as observed for 1 indicate the existence of a solution conformation for the *trans*-decalin system of 1 in benzene-d_6 which resembles the

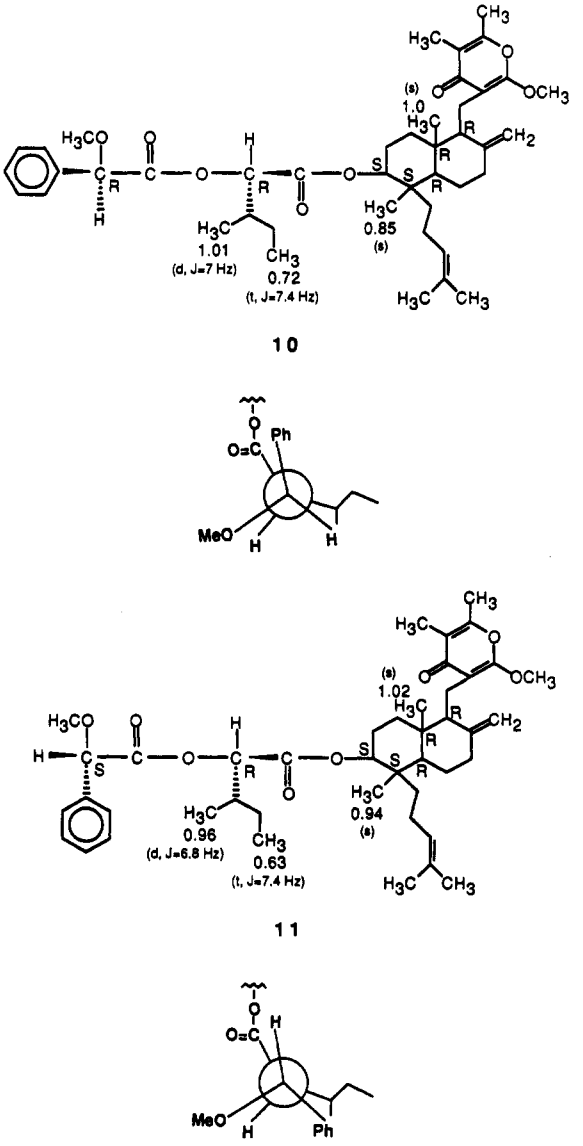


Figure 3. Selected ^{1}H NMR chemical shifts of the *R*/*S* pair of *O*-methyl mandelates of 1 and their extended Newman projections.

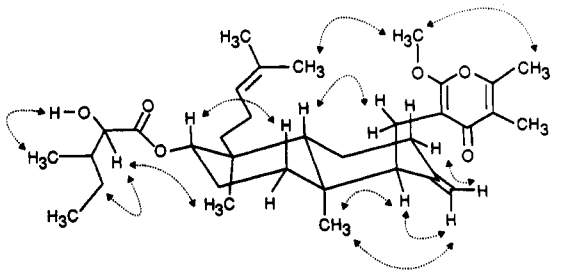


Figure 4. NOESY connectivities and solution conformation of 1 in benzene-d_6.

crystal conformation of 3 (Figure 2). The observation of NOESY interactions between C-15 methyl and C-9′ methyl protons with the C-7′ methoxyl protons can probably be explained by the existence of a dynamic equilibrium resulting from free rotation around the C-2′–O(CH_3) bond.

Reported biosynthetic studies on colletotrichins (7–9) in *C. nicotianae* using differently labeled formate, acetate, and mevalonate established that the terpenoid moiety is derived from acetate–mevalonate–geranyl–geranyl pyrophosphate route (incorporation of 4 mevalonate units

(12) Goddard, R.; Hatton, I. K.; Howard, J. A. K.; MacMillan, J.; Gilmore, C. J. *J. Chem. Soc., Chem. Commun.* **1976**, 408. Gohbara, M.; Kosuge, Y.; Suzuki, A.; Tamura, S.; Ohashi, Y.; Sasada, Y. *Acta Crystallogr.* **1977**, *B33*, 1276.

(13) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512. Trost, B. M.; Belletire, J. L.; Godleski, S.; McDougal, P. G.; Balkovec, J. M.; Baldwin, J. J.; Christy, M. E.; Ponticello, G. S.; Varga, S. L.; Springer, J. P. *J. Org. Chem.* **1986**, *51*, 2370.

(14) Bodenhausen, G.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *106*, 1304. The NOESY data were acquired on a Varian XL-400 NMR spectrometer (400 MHz) with a 125 mM solution of 1 in benzene-d_6. FIDs were acquired without spinning the sample with a mixing time of 200 ms.

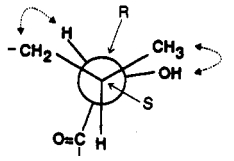


Figure 5. Newman projection across C-3″–C-2″ bond in 1 (double-headed arrows indicate NOESY connectivities).

consistent with labeled acetate incorporations) and that the γ-pyrone moiety is of acetate-polyketide origin.[15] Biosynthesis of the diterpenoid–γ-pyrone moiety of viridoxins can also be expected to involve similar intermediates (without the loss of a carbon from one of the mevalonates which is required for the formation of the aliphatic side chain C-11–C-15 of the colletotrichins). α-Hydroxy acids required for the esterification of this diterpene–γ-pyrone alcohol to viridoxins can be derived by reduction of α-keto acids (α-ketoisocaproic acid and α-keto-β-methylvaleric acid for **3** and **1**, respectively), intermediates in amino acid biosynthesis (leucine and isoleucine respectively).[16] Esterification of the alcohol, presumably the last step in the biosynthesis of viridoxins in *M. flavoviride*, apparently is catalyzed by an enzyme (acyl transferase), which is missing in *C. nicotianae*. **5** is the key precursor in the biosynthesis of **1**, **3**, and **7–9** as the side chain C-11–C-16 of **5** can rearrange with the loss of C-13 to furnish the C-11–C-15 moiety of **7–9**.[15] 8′-CH_3 in **5** can be oxidized to an acid followed by esterification to give rise to the $-COOCH_3$ (C-7′–C-8′) functionality in **7–9**. It is unusual that two unrelated entomopathogenic and phytopathogenic fungi produce toxins which have common basic structures but apparently have different biological roles. Hydrolysis of viridoxins leads to a complete loss of insect toxic properties but at the same time the product alcohol becomes a phytotoxin. There seems to be only one precedence for this where the entomopathogen *M. anisopliae* and the phytopathogen *Alternaria brassicae* both produce the cyclodepsipeptide toxin, destruxin B, as the bioactive metabolite.[17]

1 and **3** are both potent insect toxins. LC_{50}s for the insect Colorado potato beetle (*Leptinotarsa decemlineata*, Coleoptera) were determined to be 39.7 ppm (95% fiducial limits 37.8–41.6 ppm) and 50.7 ppm (limits 45.4–55.3 ppm), respectively, for **1** and **3**. **2** and **6** had LC_{50}s of 39.6 ppm (limits 35.7–43.4 ppm) and 76.6 ppm (limits 67.9–84.7 ppm), respectively. The bioassays were carried out using a leaf-disk assay as previously reported, and the LC_{50}s were calculated by Probit analysis.[18] Interestingly, the hydrolysis product of viridoxins, **5**, which is the phytotoxin colletochin, did not show any insect toxicity even at a dosage of 1000 ppm. The data suggest that the α-hydroxy acid moiety of viridoxins is important for their biological activity. The mode of action of viridoxins (**1** and **3**) is presently not known. In the case of colletotrichins (**7–9**), it has been demonstrated that they inhibit the oxidation of succinate and substrates with NAD-linked dehydrogenases by the rat liver mitochondria and inhibit mitochondrial respiration.[19]

Experimental Section

Culturing of the Fungus, Extraction, and Isolation of Viridoxins A (1) and B (3). The isolate of the fungus *M. flavoviride*, used in the present studies, was obtained from the USDA-entomopathogenic fungal collection located at Ithaca (isolate no. ARSEF 2133). The fungus was originally isolated in 1956 from *Ceutorrhynchus macula-alba* (Coleoptera) in Czechoslovakia. The culture was maintained at 5 °C on slants of Sabouraud Dextrose Agar. For toxin production, the fungus was cultured in liquid Sabouraud Dextrose medium (six × 1-L Fernbach flasks, 10% inoculum from a microculture) on a gyrotary shaker (110 rpm) at room temperature. The culture was harvested after 10 days of inoculation. The broth was filtered under suction over several layers of cheese cloth and the mycelium rinsed with water. The mycelial pellet was soaked in ethanol overnight, blended, and filtered over filter paper (Whatman no. 1) under suction. The clear filtrate was evaporated to dryness in vacuo and the residue resuspended in water and extracted several times with methylene chloride. The combined organic layer was washed with water and dried over anhydrous sodium sulfate and solvent removed to afford crude extract. This extract showed toxic activity against the insect Colorado potato beetle (*Leptinotarsa decemlineata*, Coleoptera). Subsequent separation and monitoring of the activity was carried out by insect toxicity bioassay using this insect according to the procedure described elsewhere.[18]

The crude methylene chloride soluble extract (1.04 g from 6 L of culture) was chromatographed on a flash silica gel column. Fractions eluted with methylene chloride–methanol (98:2) were combined, and solvent was removed to afford a residue which was redissolved in methanol and subjected to semipreparative HPLC under the following conditions: column, RP C_{18} (IBSIL, Phenomenex, 10 × 250 mm); solvent, acetonitrile–water (70:30) to acetonitrile (10 min, linear gradient); flow, 5.5 mL/min; detection, UV 220 nm. Two peaks at retention times of 13.6 and 14.3 min were pooled and solvent removed to afford viridoxins B (**3**) (0.2% yield of the methylene chloride soluble crude extract) and A (**1**) (1.1% yield), respectively. For analytical purposes, the silica gel purified toxins could be separated under the following HPLC conditions: column, RP C_{18} (IBSIL, Phenomenex, 4.6 × 250 mm); solvent, acetonitrile–water (70:30) to acetonitrile (10 min, linear gradient); flow, 2 mL/min; detection, UV 220 nm; retention times for **1** and **3**, 14.3 and 13.5 min, respectively.

Viridoxin A (1): colorless needles from acetonitrile–water at 5 °C; mp 65–66 °C; $[\alpha]^{21}_D$ −36.5° (*c* 1.51, chloroform); UV λ_{max} (MeOH) 202 (log ε 4.3), 258 nm (log ε 3.9); EIMS 556 (M^+), 541, 525, 473, 442, 425, 409, 393, 355, 343, 327, 315, 257, 235, 221, 207, 167, 153, and 113; HRFABMS 557.3834 (M + H^+, calcd for $C_{34}H_{53}O_6$ 557.3842, Δ mmu 0.8); ^1H and ^{13}C NMR data are listed in Tables I and II, respectively.

Viridoxin B (3): colorless rods from acetonitrile–water at 5 °C; mp 105–106 °C; $[\alpha]^{21}_D$ −31.7° (*c* 0.65, chloroform); UV λ_{max} (MeOH) 202 (log ε 4.2), 256 nm (log ε 3.8); HRFABMS 557.3853 (M + H^+, cacd for $C_{34}H_{53}O_6$ 557.3842, Δ mmu 1.1); ^1H and ^{13}C NMR data are listed in Tables I and II, respectively.

Acetylation of 1 to 2. A solution of 2 mg of **1** in dry pyridine (100 μL) and acetic anhydride (100 μL) was sealed under dry nitrogen and allowed to stand at room temperature for 24 h. The solvent was then removed first with a stream of nitrogen and then vacuum desiccation to afford a residue which was redissolved in methanol and subjected to HPLC under the conditions as reported above for **1**. The major peak at retention time of 15.6 min was pooled and solvent removed to obtain **2** (1.2 mg); FABMS 599 (M + H^+).

Acetylation of 3 to 4. **3** (2 mg) was acetylated and the product purified by HPLC under identical conditions as **2** above to afford **4** (1.3 mg); FABMS 599 (M + H^+).

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Hydrolysis of 1 and 3 to 5. 1 (7.1 mg) was dissolved in methanol (300 μL) and treated with 150 μL of 2 M KOH solution. The mixture was stirred at room temperature for 24 h and the solvent removed in vacuo. The residue was dissolved in water (2 mL) and extracted with methylene chloride (4 × 2 mL). The combined methylene chloride layer was washed with water and dried (anhydrous sodium sulfate) and solvent removed in vacuo. The residue (5.4 mg) was subjected to HPLC under the conditions used for the purification of 1. The major peak with a retention time of 8.9 min was collected and solvent removed in vacuo to afford 5 (3.3 mg): UV λ_{max}(MeOH) 202 (log ϵ 4.2), 257 nm (log ϵ 3.8); FABMS 443 (M + H$^+$); ^{1}H and ^{13}C NMR data for 5 are listed in Tables I and II, respectively. 3 was hydrolyzed to 5 in an identical fashion.

Acetylation of 5 to 6. 5 (2.8 mg) was treated with dry pyridine (100 μL) and acetic anhydride (100 μL) and sealed under nitrogen. The solution was allowed to stand at room temperature for 24 h, and then the solvent was removed by a stream of nitrogen followed by vacuum drying. The residue was dissolved in methanol and subjected to HPLC under the conditions as for 1. The major peak at a retention time of 12.9 min was pooled and solvent removed in vacuo to furnish 6 (1.3 mg); FABMS 468 (M + H$^+$).

(R)-*O*-Methylmandelic Acid Derivative of 1 (10). To a solution of 1 (7.44 mg, 0.013 mmol) and (*R*)-*O*-methylmandelic acid (2.23 mg, 0.013 mmol) in 2 mL of dry THF at room temperature were added 2-hydroxybenzotriazole (1.88 mg, 0.012 mMol), pyridine (0.97 mg, 0.012 mmol), and DCC (3.3 mg, 0.016 mmol) in that order. The mixture was stirred at room temperature for 48 h under dry nitrogen. An additional 2 mL of THF was added, and the solution was filtered to remove precipitated DCU. From the clear filtrate, solvent was removed in vacuo and the residue was suspended in water (3 mL) and extracted with methylene chloride (5 × 2 mL). The combined organic layer was washed with 10 mL of 10% aqueous sodium bisulfate, 10 mL of aqueous potassium carbonate, and 10 mL of water and dried over anhydrous sodium sulfate. The solvent was removed in vacuo, and the residue was dissolved in methanol and subjected to HPLC under the conditions as above for 1. The major peak at a retention time of 16.9 min was pooled and solvent removed to afford 10 (3.3 mg); FABMS 705 (M + H$^+$). The HPLC also showed a minor peak at retention time of 14.3 min which was due to the presence of some unreacted 1 in the reaction product.

(*S*)-*O*-Methylmandelic Acid Derivative of 1 (11). The derivatization was done under identical conditions as above for 10 except that (*R*)-*O*-methylmandelic acid was replaced with (*S*)-*O*-methylmandelic acid (7.44 mg, 0.013 mmol). The product was purified by HPLC under similar conditions to afford 11 (retention time 16.9 min) (4.2 mg); FABMS 705 (M + H$^+$). This reaction mixture also showed the presence of some unreacted starting material 1 (peak at a retention time of 14.3 min). The ^{1}H NMR spectra of 10 and 11 were acquired in benzene-d_6 with tetramethylsilane as internal reference (0 ppm). Selected chemical shifts for 10 and 11 are given in Figure 4.

Single-Crystal X-ray Diffraction Analysis of Viridoxin B (3). Single crystals in the form of transparent colorless rods were obtained by diffusing water into an acetonitrile solution of viridoxin B. A crystal of dimensions 0.2 × 0.3 × 0.5 mm was mounted in a capillary with mother liquor, and data were collected at room temperature on a Siemens R3M diffractometer to $2\theta \leq 116°$ (Cu K_α) using a $\theta - 2\theta$ scan with a variable scan speed. Photographs displayed only triclinic symmetry with accurate cell constants, determined by a least-squares fit of 25 reflections with measured 2θ values in the range of $30° \leq 2\theta \leq 45°$, of a = 7.259 (1) Å, b = 10.843 (2) Å, c = 11.449 (1) Å, with angles α = 66.770 (1)°, β = 78.550 (1)°, and γ = 77.890 (1)°. The space group is $P1$ with one molecule of $C_{34}H_{52}O_6$ per asymmetric unit and with a calculated density of 1.15 g/cm^3. Periodically monitored reflections showed no significant decomposition. A total of 3397 reflections was collected of which 2784 (82%) were considered observed with $|F_o| \geq 4\sigma|F_o|$. Reflections were corrected for Lorentz and polarization effects, but there was no absorption correction. The structure was solved using the SHELXTL library of programs. Full-matrix least-squares refinements with anisotropic non-hydrogen atoms and fixed riding hydrogens converged to a final residual factor of R = 0.054 and R_w = 0.063 with $w = \sigma^2(F + 0.001F^2)$. The final difference electron density map was featureless with ±0.32 e Å^3. Additional crystallographic details have been deposited at the Cambridge Crystallographic Data Centre.

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Supplementary Material Available: ^{1}H and ^{13}C NMR spectra of 1, 3, and 5 and NMR spectra of 1 in deuterated chloroform (HOM2DJ resolved) and benzene-d_6 (COSYPS, COSY-45, HETCOR, DEPT, COLOC, and NOESY) (26 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information. Tables of crystal data collection, solution and refinement parameters, fractional coordinates, bond distances and angles, and thermal factors for 3 (8 pages) can be obtained from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB21EZ, UK.