Pergamon

Tetrahedron Letters, Vol. 35, No. 51, pp. 9597-9600, 1994
Elsevier Science Ltd
Printed in Great Britain
0040-4039/94 $7.00+0.00

0040-4039(94)02134-1

Sorokinianin: a Novel Phytotoxin Produced by the Phytopathogenic Fungus *Bipolaris sorokiniana*

Hiromitsu Nakajima*, Keiko Isomi and Takashi Hamasaki

Department of Bio-resource Science, Faculty of Agriculture, Tottori University, Koyama, Tottori 680, Japan

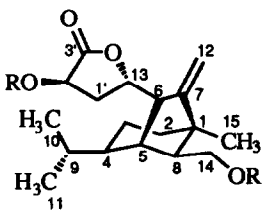
Masakatsu Ichinoe

National Institute of Hygienic Sciences, Setagaya-ku, Tokyo 158, Japan

Abstract: Structure of sorokinianin, a novel phytotoxin from an isolate of *Bipolaris sorokiniana* was determined to be 3-hydroxy-5-(8'-hydroxymethyl-4'-isopropyl-1'-methyl-7'-methylenebicyclo[3.2.1]oct-6'-yl)tetrahydro-2-furanone predominantly on the basis of 2D NMR. The preliminary incorporation study suggested that this phytotoxin is derived biogenetically not from a geranylgeranylpyrophosphate through removal of two carbons but from a farnesylpyrophosphate and an additional C_3 unit.

In the course of our investigations of phytopathogenic fungi as sources and/or metabolism models for novel phytotoxic secondary metabolites,[1] a culture of *Bipolaris sorokiniana* isolated from imported barley grain (*Hordeum vulgare* L., from Australia) was found to produce an unknown phytotoxin which inhibits germination of barley seeds. *Bipolaris sorokiniana* (Sacc.) Shoem. (anamorph of *Cochliobolus sativus* (Ito & Kurib.) Drechsler ex Dastur) is a phytopathogen which causes spot blotch or foot and root rot diseases of wheat, barley, oats and other grasses.[2] The production of sesquiterpenes,[3] sterigmatocystin[4] and bipolaramide[5] by this species of fungus has been reported. Victoxinin, a novel nitrogen-containing sesquiterpene, is the only phytotoxic metabolite reported from this species.[6] We would now like to report here on the structure and presumed biosynthetic origin of a novel phytotoxin, sorokinianin isolated from *B. sorokiniana* OB-25-1.

Sorokinianin (**1**) was isolated as an oil from the culture filtrate of the fungus, strain OB-25-1, by several chromatographies in yields of 2 mg/L of medium.[7] The ^{13}C NMR data (Table 1) and HREIMS[8] revealed its molecular formula as $C_{18}H_{28}O_4$ (5 unsaturations). The DEPT data demonstrated that two of the protons in the molecule are bound to oxygen. Acetylation of sorokinianin afforded a diacetate derivative (**2**),[9] confirming that the two exchangeable protons were associated with alcoholic hydroxy groups. A γ-lactone ring was indicated by a carbon signal at 177.2 ppm and IR carbonyl absorption at 1773 cm^{-1}. The two carbon signals at 108.1 and 160.4 ppm indicated one double bond and thereby 3 rings in the structure.



1 R = H
2 R = Ac

The structural fragments in sorokinianin shown in Fig. 1 were deduced through analysis of ^{1}H-^{1}H COSY and HETCOR, and chemical shift considerations. In the ^{1}H NMR spectrum of the diacetate (Table 1), proton signals associated with an oxygenated methine and an oxymethylene unit were shifted downfield (from 4.26 to 5.24 ppm and 3.02 and 3.58 to 3.63 and 3.94 ppm), indicating that the two carbons (C-2' and C-14) in natural product bear free OH groups.

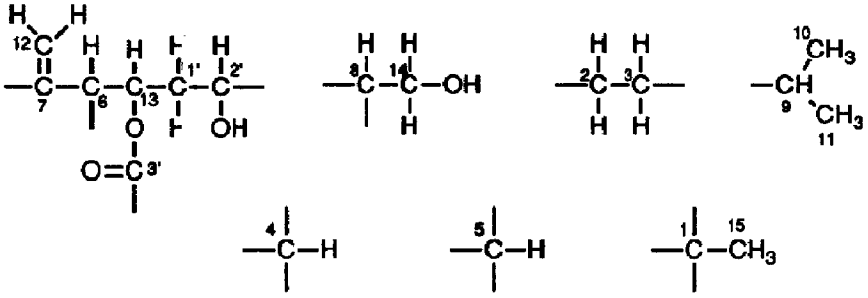


Fig.1 Structural Fragments of Sorokinianin (**1**)

The connectivity of these structural fragments was deduced by long-range ^{13}C-^{1}H correlations detected through an LR-HETCOR experiment (Table 1). Three carbons, C-2, C-7 and C-8, as well as C-1 correlated with 15-H_3, indicating the connections of C-1 with C-2, C-7 and C-8. The connections of C-5 with C-6 and C-8 were revealed by correlations of three carbon resonances for C-1, C-7 and C-14 with 5-H proton signal. The connections described thus far were confirmed by the correlations between C-7 and 8-H, C-1 and 12-H_2, and C-7 and H-8. The carbon resonance for C-4 showed correlations to the proton signals due to two geminal methyls (10-H_3 and 11-H_3), indicating the connection of C-4 and C-9. The C-3' signal correlated with the 1'-H_2 signal and the C-3 signal correlated with the 5-H signal. These lead to the plane structure for sorokinianin.

The relative stereochemistry of sorokinianin is proposed on the basis of the ^{1}H NMR coupling constant and NOE difference spectral data. For example, the coupling constants of the γ-lactone ring protons ($J_{2,1'}$=3.6, 7.1 Hz and $J_{1',13}$=6.0, 7.1 Hz) indicated the *trans* orientation of two substituents of the ring.[10] The key NOE difference spectral data as well as ^{1}H NMR coupling constants suggested the relative stereochemistry of sorokinianin as shown in Fig. 2. Additionally, the MM-2 energy calculation afforded the most stable conformational model for sorokinianin (Fig. 2), which coincided perfectly with NOE results and ^{1}H NMR coupling constants.

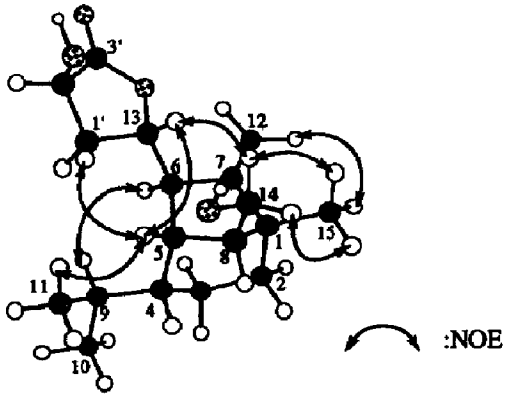


Fig. 2 Significant NOE Correlations and the Most Preferable Conformation (35.7 kcal/mol) in Sorokinianin

Table 1. NMR (^{1}H, 270.05 MHz, ^{13}C, 67.8 MHz) Data for Sorokinianin and Its Acetyl Derivative

Position	Sorokinianin (1)			Acetyl derivative (2)	
	δ_C	δ_H	LR-HETCOR	δ_C	δ_H
1	48.8		5-H, 12-H_2, 15-H_3	47.8	
2	44.2	1.16[a], 1.39[a]	15-H_3	42.6	0.97–1.67[d]
3	25.8	1.01[a], 1.56[a]	5-H	24.3	0.97–1.67[d]
4	50.9	0.97[a]	10-H_3, 11-H_3	49.4	0.97–1.67[d]
5	39.2	2.30 br.s		39.2	2.07 br.s
6	49.9	2.36 br.d (10.4)	12-H_2, 1'-Ha[b]	48.1	2.52 br.d (10.3)
7	160.4		5-H, 8-H, 15-H_3	156.4	
8	58.5	1.49[a]	2-Ha[b], 15-H_3	53.8	0.97–1.67[d]
9	31.2	1.30[a]	10-H_3, 11-H_3	30.1	0.97–1.67[d]
10	21.3	0.73 d (6.6)	11-H_3	20.5[c]	0.75 d (6.4)
11	22.6	0.87 d (6.6)	10-H_3	20.8[c]	0.87 d (6.4)
12	108.1	4.73 br.s		109.2	4.81 br.d (2.0)
		5.20 br.s			5.27 br.d (1.0)
13	83.6	4.45 ddd		82.5	4.41 ddd
		(6.0, 7.1, 10.4)			(6.8, 6.8, 10.3)
14	63.1	3.02 dd (10.9, 10.9)	5-H, 8-H	65.0	3.63 dd (9.3, 11.5)
		3.58 dd (6.6, 10.9)			3.94 dd (7.1, 11.5)
15	21.1	0.89 s		21.5	0.95 s
1'	38.7	2.05 ddd	2'-H	34.8	2.30 m
		(7.1, 7.1, 13.7)			
		2.24 ddd			
		(3.6, 6.0, 13.7)			
2'	68.8	4.26 dd (3.6, 7.1)	13-H, 1'-Hb[b]	68.4	5.24 dd (4.9, 6.8)
3'	177.2		1'-Ha[b]	172.1	
OH		3.51, 4.96			
*C*OCH$_3$				169.7	
				170.7	
CO*CH*$_3$				20.2[c]	1.88 s
				20.3[c]	1.96 s

[a] overlapping NMR signals; [b] Ha means the methylene proton that resonates in the upper field;
[c] may be interchanged; [d] overlapping NMR signals; abbreviations, s: singlet, d: doublet, br.: broad.

Sorokinianin is a novel fungal metabolite composed of a C_3 unit plus the same carbon skeleton as prehelminthosporol,[11] which is a sesquiterpene and was found to be a major metabolite of this fungus. There are two possible biosynthetic routes to generate sorokinianin: one from a sesquiterpene and a C_3 unit, and another from a diterpene through removal of a C_2 unit or two C_1 units. The former is more likely for the following reason. We have never detected any diterpene among the metabolites of this fungus although this fungus produces many sesquiterpenes, such as prehelminthosporol, helminthosporol and helminthosporic acid (data not shown). Furthermore, in the replacement culture this fungus was incubated with prehelminthosporol and produced six times more sorokinianin (2.1 mg/L) than the control without prehelminthosporol (0.35

mg/L).[12] Experiments designed to determine the biosynthetic origin of sorokinianin ambiguously are currently in progress.

One mM of sorokinianin inhibited germination of barley seeds (*H. vulgare* L.) completely and 300 μM of this compound reduced their germination ratio to 50%. In the protoplast bioassay[13] 300 μM of sorokinianin killed all protoplasts obtained from the first leaves of barley and the IC_{50} value of this compound in this bioassay was estimated to be 150 μM. The acetyl derivative was virtually inactive in the protoplast bioassay, indicating involvement of hydroxyl groups in the mechanism of its phytotoxicity.

References and Notes

1. (a) *Cochliobolus spicifer* : Nakajima, H.; Fujimoto, H.; Matsumoto, R.; Hamasaki, T. *J. Org. Chem.* **1993**, *58*, 4526 and references cited therein. (b) *Neocosmospora vasinfecta* : Nakajima, H.; Fukuyama, K.; Kimura, Y.; Hamasaki, T. *Biosci. Biotech. Biochem.* **1992**, *56*, 1148 and references cited therein.
2. Sivanesan, A. *Mycological Papers, No. 158. Graminicolous Species of Bipolaris, Curvularia, Drechslera, Exserohilum and their Teleomorphs;* C•A•B International Mycological Institute: Kew. 1987; pp. 62-65.
3. (a) Dorn, F.; Arigoni, D. *Experientia* **1974**, *30*, 851. (b) Dorn, F.; Arigoni, D. *Ibid.* **1975**, *31*, 753. (c) Nukina, M.; Hattori, H.; Marumo, S. *J. Am. Chem. Soc.* **1975**, *97*, 2542. (d) Winter, R. E. K.; Dorn, F.; Arigoni, D. *J. Org. Chem.* **1980**, *45*, 4786.
4. Rabie, C. J.; Lübben, A.; Steyn, M. *Appl. Environ. Microbiol.* **1976**, *32*, 206.
5. (a) Maes, C. M.; Steyn, P. S.; van Rooyen, P. H.; Rabie, C. J. *J. Chem. Soc., Chem. Commun.* **1982**, 350. (b) Maes, C. M.; Steyn, P. S.; Vleggaar, R.; Kirby, G. W.; Robins, D. J.; Stark. W. M. *J. Chem. Soc. Perkin Trans I* **1985**, 2489.
6. (a) Dorn, F.; Arigoni, D. *J. Chem. Soc., Chem. Commun.* **1972**, 1342. (b) Pringle, R. B. *Can. J. Biochem.* **1976**, *54*, 783.
7. The fungus was grown on a medium containing glucose (50 g/L), peptone (3 g/L), the extract from 50 g/L of malt and water at 24°C without shaking. After 14 days incubation the cultures were filtered and the culture filtrate was treated twice with activated charcoal at pH 2.0. The metabolites were extracted from the activated charcoal with acetone and the solvent fractionation of the acetone extract with EtOAc gave EtOAc-soluble neutral fraction. Silica gel column chromatography (30% acetone in *n*-hexane) and Sephadex LH-20 column chromatography (MeOH) of the fraction and subsequent purification by silica gel flash chromatography (40% and 50% EtOAc in *n*-hexane) gave sorokinianin.
8. 1: colorless oil; HREIMS: found *m/z* 308.2019, calcd for $C_{18}H_{28}O_4$ *m/z* 308.1988; $[\alpha]_D^{20}$ +46° (*c* 1.0, MeOH); FTIR ν_{max} (film): 3328, 1773, 1183, 1127 cm^{-1}; UV λ_{max} (EtOH): 202 nm (ε 3000).
9. 2: colorless oil; HREIMS: found *m/z* 392.2203, calcd for $C_{22}H_{32}O_6$ *m/z* 392.2199; $[\alpha]_D^{20}$ +40° (*c* 0.5, $CHCl_3$); FTIR ν_{max} (film): 1794, 1748, 1655, 1230 cm^{-1}.
10. Maier, M. E.; Kandler, H.; Haller, B. U.; Hofman, J. H.; Fischer, H. *Libigs Ann. Chem.* **1990**, 323.
11. Pena-Rodriguez, L. M.; Armingeon, N. A.; Chilton, W. S. *J. Nat. Prod.* **1988**, *51*, 821.
12. The fungus was grown on 1 ml of medium in a test tube at 24°C without shaking. After 7 days incubation the medium was replaced with 1 ml of new phosphate buffer (pH 7.5) and the fungus was incubated for 24 hr. Then the buffer was replaced with 1 ml of new phosphate buffer with or without 1 mg of prehelminthosporol and the fungus was incubated for 3 days. The converted products were extracted with EtOAc from the culture filtrate and the amount of sorokinianin was determined after acetylation of the extract by capillary GC-SIM monitored at *m/z* 115 and 143, which are the prominent fragment ions in EIMS of acetyl derivative. GC-SIM conditions: column: HP-5 (30 m x 0.32 x 0.25 μm film thickness); oven temperature: 240°C; flow rate of He: 1.7 ml/min; interface temperature: 200°C; ion source temperature: 180°C; ionization voltage: 70 eV.
13. Nakajima, H.; Kimura, Y.; Hamasaki, T. *Phytochemistry* **1992**, *31*, 105.

(*Received in Japan* 5 *August* 1994)