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Lecythophorin, a Potent Inhibitor of Blue-stain Fungi, from the Hyphomycetous Fungus *Lecythophora hoffmannii*.

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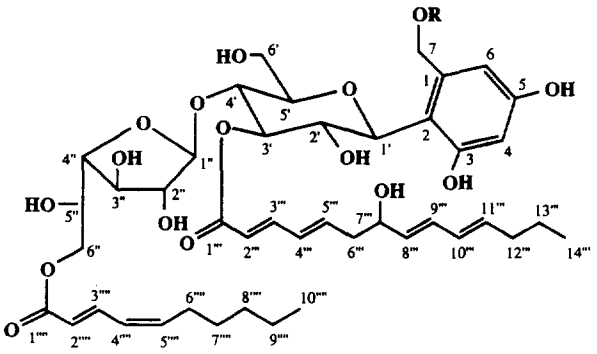
Abstract: A novel chaetiacandin sulfate, lecythophorin (**1**), has been isolated from the hyphomycetous fungus *Lecythophora hoffmannii*. The structure was determined by a combination of chemical and spectroscopic methods. This sulfate is strongly antagonistic to the aspen staining fungus *Ophiostoma crassivaginatum*.

Aspen (*Populus tremuloides* Michaux) is a widely distributed tree species in North America. Commercially it is one of the most important species of hardwood. The decay and staining of aspen caused by fungi, especially the so-called blue-stain fungi, is a serious problem associated with aspen utilization in Canada.[1,2] In the course of investigating methods for biological control of blue-stain fungi of aspen, Hiratsuka *et al.* found that *Lecythophora hoffmannii* (van Beyma) W. Gams and McGinnis, a hyphomycetous fungus, is strongly antagonistic to *Ophiostoma crassivaginatum* (H.D. Griffin) T.C. Harrington, one of the most common blue-stain fungi on *P. tremuloides*.[3] Bioassay-guided purification of a crude extract of *L. hoffmannii* resulted in the isolation of a novel chaetiacandin[4] sulfate, which we have designated lecythophorin (**1**). The structural elucidation of compound **1** is reported in this paper. A revised structure **2** is suggested for chaetiacandin.

The fungus was isolated from healthy wood of *P. tremuloides*[3] and grown on sterilized rice medium at 23° (2 kg, presoaked with water) for three weeks. The acetone extract of the rice medium, which showed antifungal activity against *O. crassivaginatum*, was chromatographed on silica gel ($CHCl_3$-MeOH, 4:1) to give a bioactive fraction. This fraction was further purified by chromatography on Sephadex LH-20 ($CHCl_3$-MeOH, 3:1) and reverse phase silica gel (MeOH-H_2O, 3:2) to afford lecythophorin (**1**, 5 mg) as an amorphous powder.

Lecythophorin (**1**), $[\alpha]_D$ +8.4° (c = 0.61, MeOH), gave peaks at m/z 957 ($[M+Na]^+$) and 973 ($[M+K]^+$) by laser mass spectrometry, and positive high resolution FAB ms determined the molecular formula to be $C_{43}H_{59}O_{19}SNa$ (m/z 957.3137 $[M+Na]^+$, Δ -3.0 mmu). The presence of a sulfate group is substantiated by a strong IR absorption at 1267 cm^{-1} and by a peak at m/z 855 ($[M-SO_3Na+H+Na]^+$) in the FAB ms. The IR spectrum of **1** suggests the presence of hydroxyl groups (3400 cm^{-1}) and unsaturated carbonyl groups (1700

and 1640 cm^{-1}) in addition to the sulfate group. The UV spectrum (MeOH) shows an absorption maximum at λ_{max} 263 nm, similar to that of chaetiacandin.[5] The ^1H NMR spectrum (Table 1) of **1** exhibits 51 non-exchangeable protons, including two methyl groups on saturated carbons, two aromatic protons with *meta*-



1 : R = SO_3Na
2 : R = H

coupling, two benzyl alcohol protons, and twelve olefinic protons. The ^{13}C NMR spectrum (Table 1) of **1** displays 2 methyls, 10 methylenes, 25 methines, 4 fully substituted aromatic carbons, and 2 ester carbonyl carbons (δ 168.8 and 169.0). Extensive analysis of 2D NMR spectra (^1H-^1H COSY, HOHAHA, HMQC, HMBC, and ROESY) suggested that **1** consisted of a dihydroxybenzyl alcohol moiety, two sugar units, and two unsaturated fatty acids.

The benzyl alcohol protons at δ 4.94 and 5.20 (H-7) show HMBC correlation to a hydrogen-bearing aromatic carbon at δ 110.1 (C-6), and to two aromatic carbons at δ 138.6 (C-1) and 115.3 (C-2), whereas an aromatic proton at δ 6.25 (H-4), which is *meta*-coupled with δ 6.40 (H-6), shows HMBC correlation to C-2, C-6, and two oxygen-bearing aromatic carbons at δ 158.6 (C-3) and 159.2 (C-5). These data indicate that **1** contains a 2-substituted 3,5-dihydroxybenzyl alcohol moiety in the molecule. The sulfate function is attached to the benzyl alcohol oxygen since the chemical shifts of the benzyl protons moved upfield (δ 4.54 and 4.62) after removal of the sulfate by treatment with dioxane-pyridine.[6]

The first sugar unit was identified as a glucopyranose by measurement of the ^1H-^1H coupling constants and the ^1H-^1H COSY spectrum. The HMBC spectrum of **1** shows cross peaks due to ^1H-^{13}C long-range correlations for δ 4.90 (H-1') to C-1, C-2, and C-3, thus indicating that C-2 of the benzene ring is connected directly to C-1' of glucose. The stereochemistry of the anomeric position of the glucopyranose is assigned as β on the basis of the ^1H-^1H coupling constant ($J_{1',2'}$ = 9.5 Hz), and the ROESY correlation between H-1' and δ 3.58 (H-5'). The alkaline hydrolysis of **1** followed by methanolysis with hydrogen chloride in methanol gave an α-D-methylgalactopyranoside, which was identified as its tetraacetate by comparison with an authentic sample. The ^1H-^1H coupling constants ($J_{1'',2''}$ = 2.5 Hz, $J_{2'',3''}$ = 4.7 Hz, and $J_{3'',4''}$ = 6.2 Hz) of **1** were different from that of a galactopyranoside.[7] From comparison of the ^{13}C NMR chemical shifts of the galactose moiety of **1** with those of the four isomers of methyl galactoside,[8,9] the second sugar unit of **1** was identified as a β-D-galactofuranose. The HMBC correlation observed for δ 5.02 (H-1''), the anomeric proton

Table 1. ^{1}H and ^{13}C NMR Chemical Shifts and Heteronuclear Multiple Bond (HMBC) Correlations of Lecythophorin (**1**) in CD_3OD.

position		^{1}H [a]	J (Hz)	^{13}C [b]	HMBC(^{1}H) [c]
1				138.6 s	6, 7, **1'**
2				115.3 s	4, 6, 7, **1'**
3				158.6 s	4, **1'**
4		6.25 d	2.4	104.8 d	6
5				159.2 s	4, 6
6		6.40 d	2.4	110.1 d	4, 7
7	(a)	4.94 d	11.4	69.4 t	6
	(b)	5.20 d	11.4		
1'		4.90 d	9.5	82.7 d	
2'		4.01 t	9.5	72.5 d	3'
3'		5.16 t	9.5	79.2 d	
4'		3.89 t	9.5	76.7 d	3', 6', 1"
5'		3.58 ddd	1.5, 4.0, 9.5	82.7 d	
6'	(a)	3.76 dd	4.0, 12.1	61.6 t	
	(b)	3.84 dd	1.5, 12.1		
1"		5.02 d	2.5	110.4 d	
2"		3.87 dd	2.5, 4,7	81.6 d	
3"		3.95 dd	4.7, 6.2	77.9 d	
4"		3.77 dd	2.6, 6.2	85.1 d	1"
5"		3.81 ddd	2.6, 4.0, 8.4	69.8 d	3", 4"
6"	(a)	4.07 dd	4.0, 11.7	67.8 t	4"
	(b)	4.13 dd	8.4, 11.7		
1'''				168.8 s	3', 2''', 3'''
2'''		5.94 d	15.4	121.4 d	3''', 4'''
3'''		7.38 dd	11.0, 15.4	146.8 d	5'''
4'''		6.29 dd	11.0, 15.4	132.0 d	2''', 6'''
5'''		6.13 dt	6.6, 15.4	141.8 d	3''', 6''', 7'''
6'''	(2H)	2.30 q	6.6	42.2 t	4'''
7'''		4.09 q	6.6	72.5 d	6''', 8'''
8'''		5.49 dd	6.6, 15.0	134.1 d	6''', 10'''
9'''		6.11 dd	10.6, 15.0	131.9 d	7''', 8''', 11'''
10'''		5.97 dd	10.6, 15.0	131.2 d	8''', 12'''
11'''		5.63 dt	7.3, 15.0	135.8 d	9''', 12''', 13'''
12'''	(2H)	2.01 q	7.3	35.8 t	10''', 14'''
13'''	(2H)	1.36 m		23.5 t	11''', 12''', 14'''
14'''	(3H)	0.91 t	7.0	14.3 q	12'''
1''''				169.0 s	6", 3''''
2''''		5.90 d	15.4	121.9 d	3'''', 4''''
3''''		7.65 dd	11.4, 15.4	141.3 d	5''''
4''''		6.15 t	11.4	127.6 d	2'''', 3'''', 6''''
5''''		5.88 dt	7.7, 11.4	143.1 d	3'''', 6'''', 7''''
6''''	(2H)	2.29 q	7.7	29.2 t	4'''', 7''''
7''''	(2H)	1.40 m		30.2 t	
8''''	(2H)	1.29 m		32.5 t	7'''', 9'''', 10''''
9''''	(2H)	1.30 m		23.5 t	7'''', 8'''', 10''''
10''''	(3H)	0.90 q	7.0	14.0 q	

[a]500 MHz. [b]125 MHz. [c]Optimized for $^{n}J_{CH} = 6$ Hz.

of the galactose, to δ 76.7 (C-4'), indicated that the galactose is attached to C-4' of glucose. The low-field resonances at δ 5.16 (H-3') of glucose and δ 4.07 and 4.13 (H-6") of galactose implied that C-3' and C-6" are acylated. HMBC connectivity between H-3' and δ168.8 (C-1'''), which is assigned to the ester carbonyl of the first unsaturated fatty acid, and H-6" and δ 169.0 (C-1''''), which is assigned to the ester carbonyl of the second unsaturated fatty acid, allowed the placement of these groups.

The first fatty acid chain contained in **1** was established as a 7-hydroxy-2,4,8,10-tetradecatetraenoyl group by analysis of the proton connectivities observed in the ^{1}H-^{1}H COSY and HOHAHA spectra of **1**. All double bonds were shown to have the *E* configuration by the large (≥ 15 Hz) ^{1}H-^{1}H coupling constants. The second fatty acid chain was similarly determined to be a 2,4-decadienoyl group by the ^{1}H-^{1}H COSY and HOHAHA spectra of **1**, and the double bonds were shown to possess *E* (15.4 Hz) and *Z* (11.4 Hz) configurations, respectively. Thus, the structure of lecythophorin is as shown in **1**.

The ^{1}H and ^{13}C NMR data of the desulfated portion of **1** are in good agreement with those recorded for chaetiacandin,[4] the second sugar unit of which was assigned as galactopyranose. This suggests that the structure of chaetiacandin should be revised to the galactofuranose **2**.[10]

Lecythophorin (**1**) exhibited inhibitory activity against the blue stain fungi *O. crassivaginatum* and *O. piliferum*, both in agar diffusion plates and on aspen wood chips at concentrations as low as 1 μg/mℓ. It showed no activity against the aspen rotting fungus *Phellinus tremulae*.[11]

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