Gloeolactone, a New Epoxy Lactone from a Blue-Green Alga

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A novel lactone, gloeolactone (**1**), has been isolated from the blue-green alga, *Gloeotrichia* sp. The structure of this compound has been elucidated from a detailed analysis of the NMR spectra. This compound was shown to be toxic to brine shrimp.

Toxic blue-green algal blooms in Montana's lakes have occasionally caused dermatitis, illness, and even death.[1] The two main culprits have been *Anabaena flos-aquae* and *Aphanizomenon flos-aquae*. The toxic components of these algae have been extensively studied and shown to be anatoxin-a and neosaxitoxin, respectively. Other freshwater and marine blue-green algae have been studied and shown to produce a wide variety of toxic substances.[2] Our laboratory has isolated a new toxin from a freshwater blue-green algal bloom.

The bluegreen alga, *Gloeotrichia* sp., was collected along the shores of Clark Canyon Reservoir, Montana, in August 1996. The alga was briefly air-dried and then extracted three times with $CHCl_3$−MeOH 1:1. The combined organic extracts showed activity against *Vibrio harveyii* and brine shrimp. It is interesting to note that in our antimicrobial screen against eight organisms, this extract showed activity against only one marine organism. Fractionation of this extract on LH-20 and reversed-phase HPLC yielded 38 mg (2.2% of the crude organic extract) of the bioactive constituent, which has been named gloeolactone, as a colorless oil.

The mass spectrum of gloeolactone (**1**) gave a molecular ion at *m/z* 292, and the molecular formula of $C_{18}H_{28}O_3$ was established by HREIMS. The carbon spectrum showed only 17 carbons in $CDCl_3$, but in CD_3OD all 18 carbons could be resolved. The carbon spectrum indicated an ester carbon, four olefinic methine carbons, a downfield oxygen-bearing methine, two relatively upfield oxygen-bearing methines assigned to an epoxide, nine methylenes, and one methyl carbon. The epoxide carbons at 57.6 and 60.0 ppm were correlated to the protons at 3.23 and 2.90 ppm, respectively, by the HMQC experiment, supporting the presence of a three-membered oxygen-containing ring. The molecular formula required five sites of unsaturation, which indicated one additional ring. The substitution of the side chain was established by consideration of the COSY and HMBC spectra and extensive decoupling experiments at 250 and 500 MHz. At 300 MHz the downfield protons could be resolved into a doublet of doublets for C10, a two-proton multiplet for the protons at C16 and C11, and a two-proton multiplet for C15 and C9. At 500 MHz these could be resolved and interpreted for all protons (see Figure 1). The *E* stereochemistry of the C10−C11 double bond was assigned by the large, 15.5 Hz vicinal coupling constant and the *Z* C15−C16 alkene assigned by the 10.5 Hz coupling. The trans epoxide stereochemistry was deduced from the small 2.0 Hz C12−C13 vicinal coupling. The final unit of unsaturation was filled by a 10-membered ring lactone. Pure gloeolactone was devoid of any activity against *V. harveyi*, but it did show weak toxicity in our brine shrimp assay (100% kill at 125 μg/mL).

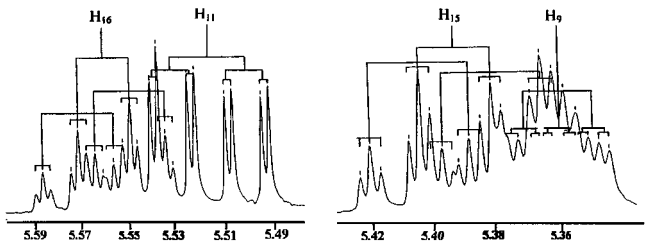
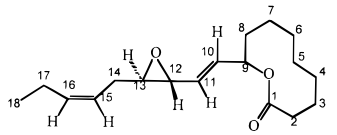


Figure 1. Downfield regions of the ^{1}H-NMR (500 MHz) spectrum of **1** with coupling analysis.



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The hydroxy acid, helenynolic acid, which has similar oxidation to the fatty-acid-derived gloeolactone**,** has been isolated from the composite *Helichrysum bracteatum*,[3] and cytotoxic epoxy-lactones have been reported from the brown alga, *Ecklonia stolonifera*.[4]

Experimental Section

General Experimental Procedures. ^{1}H- and ^{13}C-NMR spectra were recorded in CD_3OD on Bruker DRX-500, DPX-300, and DRX-250 spectrometers. ^{1}H-NMR spectra were recorded at 500 MHz, and the ^{13}C-NMR spectra were recorded at 67.5 MHz. The HMQC and HMBC spectra were run at 250 MHz with a gradient probe using standard pulse programs. All of the chemical shifts were recorded with respect to the chemical shift of the deuterated solvent. The IR spectrum was recorded on a Perkin−Elmer 1310 spectrometer. The optical rotation was recorded on a Perkin−Elmer 241 MC polarimeter using a 1-mL cell. The mass spectra were provided by the Montana State Mass Spectrometer facility at Montana State University. All solvents used were spectral grade.

Algal Material. *Gloeotrichia* J. G. Agardh, 1842. (***gloeo-*** in Gk. comp., glue-, sticky; ***trich-*** in Gk. comp.,

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Table 1. ^{1}H-NMR (500 MHz) and ^{13}C NMR (75 MHz) Data for Gloeolactone, (**1**) [CD_3OD, δ in ppm and J in Hz]

carbon	ppm (m)	proton (m, J Hz)[a]	COSY	HMBC
1	174.2 (s)			
2	34.9 (t)	2.59 (ddd, 15.5,7.5,3.0)	2.15	C−1
		2.15[a]	2.59	C−1
3	20.6 (t)			
4	23.8 (t)			
5	23.5 (t)			
6	24.3 (t)			
7	27.2 (t)			
8	30.0 (t)	2.05 (ddt, 16.5,9.0,3.5)	5.36, 1.5	C−9
		1.5[b]	5.36, 2.05	C−9
9	75.6 (d)	5.36 (tdd,5.5,3.5,1.5)	6.03, 2.05,1.5	C−10
10	134.7 (d)	6.03 (dd, 15.5, 5.5)	5.52, 5.36	C−8, C−9, C−11, C−12
11	129.1 (d)	5.52 (ddd,15.5,8.0,1.5)	6.03, 3.23	C−9, C−10, C−12, C−13
12	57.6 (d)	3.23 (dd, 8.0, 2.0)	5.52, 2.90	C10, C−11, C−13, C−14
13	60.0 (d)	2.90 (dt, 5.5, 2.0)	3.23, 2.37	C−11, C−12, C−14
14	29.5 (t)	2.37 (br, 6.0)	5.39, 2.90	C12, C−13, C−15, C−16
15	122.6 (d)	5.39 (dtt,10.5,7.0,1.5)	5.56, 2.37	C−16
16	133.0 (d)	5.56 (dtt,10.5,7.5,1.5)	5.39, 2.09	C−17, C−18
17	20.7 (t)	2.09 (pd,7.5,1.5)	5.56, 5.39, 0.99	C−15, C−16, C−18
18	13.5 (q)	.99 (t, 7.5)	2.09	C−16, C−17

[a] Obtained from the HMQC experiment [b] Estimated from the HMQC and COSY experiments.

hairy or hair-like) is a gelatinous genus of cyanobacteria, conspicuously vaginate and falsely branched; the broad, transversely plicate sheath is encompassed by a firm, spherical mucus, and the sheath may also be basally succate. The uniseriate trichome is radiate, two to several per sheath, and it is regularly attenuated from the base to apex. This genus of cyanobacteria is distinctive from the similar genus *Rivularia* Roth 1797 in that it is consistently characterized by large, subterminal, elongate akinetes frequently in short catenate series separated by intervening vegetative cells, and the trichome usually has terminal or occasionally intercalary heterocysts. *Gloeotrichia* occurs in freshwater environments and can be found as either sessile or free-floating in its habit. Reproduction is by germination of akinetes or by the fragmentation of hormogonia at the attenuated end. Three species have been reported for Montana:[5] *G. echinulata* (Smith) Richter, *G. natans* (Hedw.) Rabenhorst, and *G. psium* (ag.) Thuret.

Extraction and Isolation. The alga was collected on the shore of Clark Canyon Reservoir, August 1996 (200 g dry wt). The alga was briefly dried and extracted with $CHCl_3$−MeOH 1:1 (3 × 200 mL). The combined organic extracts (1.72 g) were first fractionated on LH-20 (MeOH) and then on C-8 reversed-phase HPLC (Rainin Dynamax-60A C8) using a 60:40 MeOH−H_2O to MeOH gradient to give 38 mg of lactone **1**.

Gloeolactone **(1)**: colorless oil (2.2% of crude extract); $[\alpha]^{25}_D$ +13.0° (*c* 0.0092, MeOH); IR (neat) γ_{max} 2950, 2920, 2860, 1718, 1245, 952 cm^{-1}; ^{1}H- and ^{13}C-NMR data see Table 1; HREIMS *m/z* $[M]^+$ 292.2038 (calcd for $C_{18}H_{28}O_3$, 292.2038); EIMS 292 (0.55) 223 (25.8) 194 (15.6) 81 (71) 69 (88) 67 (100) 55 (96).

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References and Notes

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