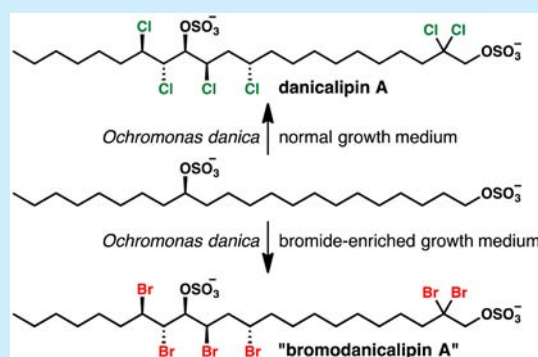


The Alga *Ochromonas danica* Produces BromosulfolipidsAlexander R. White,[†] Brendan M. Duggan,[‡] Shiou-Chuan Tsai,^{*,†,§} and Christopher D. Vanderwal^{*,†}[†]Department of Chemistry, University of California, 1102 Natural Sciences II, Irvine, California 92617, United States[‡]School of Pharmacy and Pharmaceutical Science, University of California, 9500 Gilman Drive, #0684, San Diego, California 92093, United States[§]Department of Molecular Biology and Biochemistry, University of California, 3205 McGaugh Hall, Irvine, California 92617, United States

S Supporting Information

ABSTRACT: Many halogenases interchangeably incorporate chlorine and bromine into organic molecules. On the basis of an unsubstantiated report that the alga *Ochromonas danica*, a prodigious producer of chlorosulfolipids, was able to produce bromosulfolipids, we have investigated the promiscuity of its halogenases toward bromine incorporation. We have found that bromosulfolipids are produced with the exact positional and stereochemical selectivity as in the chlorosulfolipid danicalipin A when this alga is grown under modified conditions containing excess bromide ion.



The chlorosulfolipids (Figure 1) are fascinating halogenated lipids that were first isolated in the late 1960s from the freshwater alga *Ochromonas danica*, but have since been observed in other species of algae and have been isolated from toxic shellfish and corals from the marine environment.¹ Elovson and Vagelos were the first to report the planar structure of the hexachlorinated lipid from *O. danica*, now known as danicalipin A (4).² Concurrently, Haines and co-workers engaged in extensive chemical and biochemical studies of the chlorosulfolipids from this alga.³ However, the configuration of danicalipin A and most of its less chlorinated congeners remained unknown until 2009, when our group in conjunction with Haines and the Gerwick group used a combination of synthetic and natural material to establish the relative and absolute configuration of danicalipin A via the Murata NMR method for *J*-based configurational analysis and the advanced Mosher ester method, respectively.⁴ That same year, the Okino group reisolated multiple chlorosulfolipids from *O. danica* and established their relative and absolute configurations.^{5,6}

In one particular review article, Haines noted that substitution of bromide for chloride in the growth medium for *O. danica* led to the observation of bromosulfolipids.^{1b} However, experimental details of this study, including the algal growth conditions and data used to identify the bromosulfolipids, were never published,⁷ and there was no suggestion of attempts to isolate the brominated compounds. To corroborate Haines's very reasonable claim—halogenases are often promiscuous with respect to chlorine and bromine⁸—we aimed to find growth conditions to favor formation of bromosulfolipids such that these compounds could be isolated

and fully characterized. We were equally interested in establishing that the positional and stereochemical selectivity of the bromination events mirrored those of the chlorinations, and in determining the similarities or differences in the solution conformation of the two.

Very recently, Carreira and co-workers disclosed synthetic solutions to both the brominated and fluorinated congeners of danicalipin A, naming them fluorodanicalipin A and bromodanicalipin A (see 5), respectively. They also showed that these compounds adopted identical solution conformations as the natural chlorosulfolipid, 4.⁹ At that point, we had isolated a derivative of bromosulfolipid 5 and were in the midst of securing the configurational and conformational data for this compound. In that light, we describe herein our orthogonal studies demonstrating that *O. danica* does indeed produce bromodanicalipin A when grown in media supplemented with bromide ion, and that this compound can be isolated (with great care) from a range of other chloro-, bromo-, and mixed chloro/bromosulfolipids.

There were two major challenges associated with this endeavor. First, finding suitable bromide-rich growth conditions in which the alga would flourish and produce a reasonable quantity of bromosulfolipids was not straightforward. With the assumption that the generation of chlorosulfolipids would be hard to completely suppress, the second and perhaps more difficult problem to overcome was isolation of the target compound in pure form. Indeed, the desired hexabrominated

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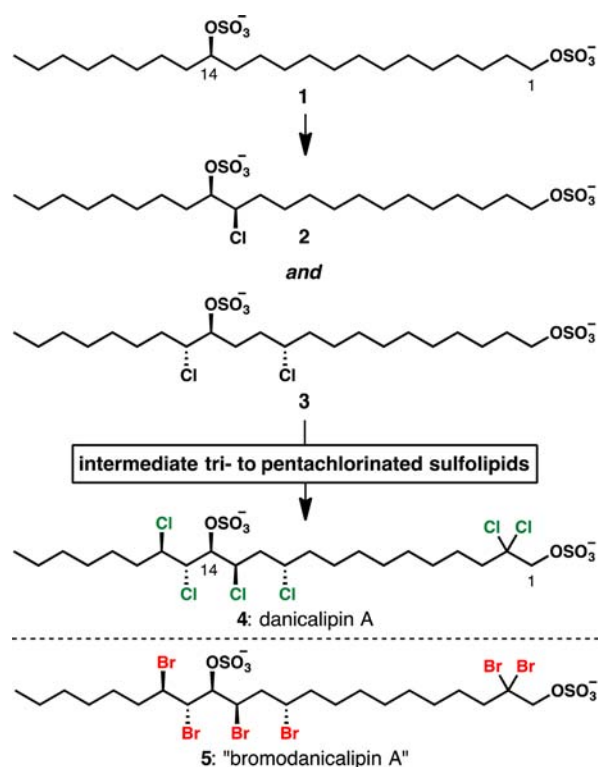


Figure 1. Currently understood sequence for chlorination in the biosynthesis of danicalipin A.

lipid would need to be separated from lipids with fewer bromides, and also from the corresponding family of chlorosulfolipids with varying degrees of chlorination. However, a further concern was the need to separate the desired hexabromosulfolipid from a potentially vast range of constitutionally isomeric lipids with each of the following overall halogen counts: Br_3Cl , Br_4Cl_2 , Br_3Cl_3 , Br_2Cl_4 , and BrCl_5 . There is certainly the potential for the generation of myriad halogenated molecules (Figure 2) expected to have very similar physical properties, from which 5 or a derivative would need to be separated.

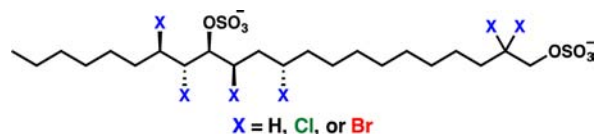


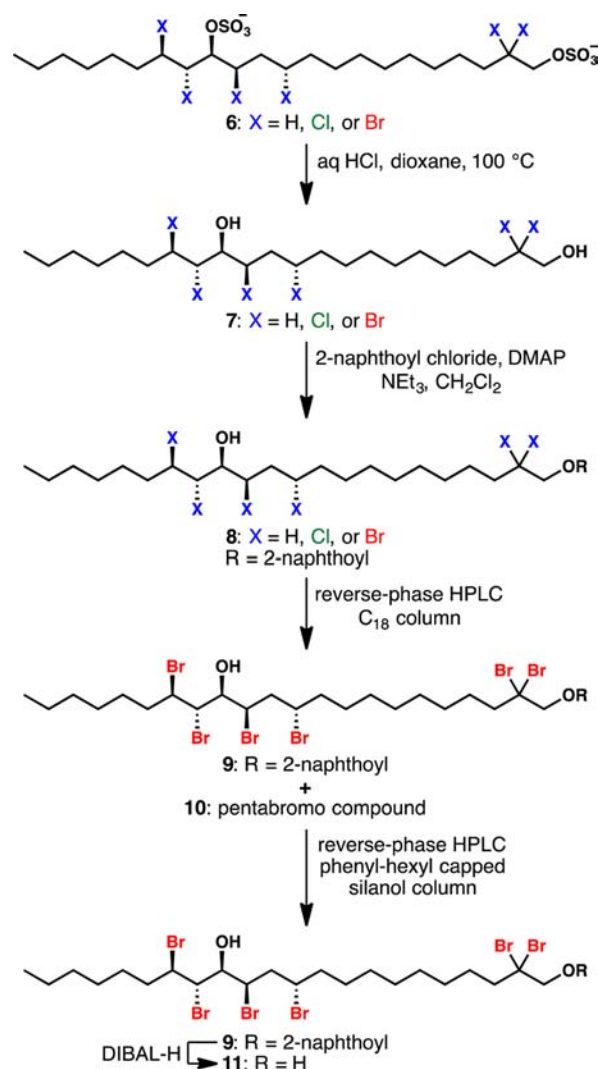
Figure 2. Potential permutations of halosulfolipids produced in bromine-enriched medium.

Our initial strategy to produce bromodanicalipin A involved utilizing a defined growth medium reported by Foster,¹⁰ but completely exchanging ammonium chloride—the major chloride source—with ammonium bromide. These conditions resulted in poor growth of the organism. However, trace quantities of bromosulfolipids, mixed chloro/bromosulfolipids, and chlorosulfolipids were detected by mass spectrometry following extraction. These promising results prompted us to optimize the algal growth conditions for bromosulfolipid production. Ultimately we determined that, rather than substantively depleting the growth medium of chloride, enrichment of the standard *Ochromonas* medium with bromide greatly enhanced production of the most highly brominated

lipid. Additionally, the overall health of the organism, as evaluated by simple visual comparison, was comparable to that of the alga cultured under standard conditions. Not surprisingly, the degree of bromine incorporation tracks reasonably well with the bromide concentration of the growth medium, and *O. danica* will tolerate up to ca. 6 g/L of ammonium bromide before growth is significantly inhibited. During the course of our study, we found that a growth medium comprising approximately 5 g/L of ammonium bromide was the optimal concentration for producing highly brominated sulfolipids and maintaining the apparent fitness of the organism.

As alluded to above, even under the optimized growth conditions, the alga still produced a convoluted mixture of bromo- and mixed-halosulfolipids (6, Scheme 1), which

Scheme 1. Production and Isolation of Bromodanicalipin A Diol (11)



rendered the isolation of our target bromosulfolipid incredibly challenging. This conundrum required us to devise a new method for their isolation and purification, because the methods reported by Okino and co-workers for danicalipin A isolation⁵ resulted, in our case, in a mixture that was inseparable by flash chromatography. Guided by the behavior of a sample of synthetic danicalipin A on TLC, the complex mixture of

halosulfolipids (6) was chromatographically isolated from the crude algal extract. We then sought to utilize HPLC to further purify this complex mixture, but the lack of a chromophore in these molecules precluded UV detection. Accordingly, the lipids were subjected to solvolysis conditions to cleave the sulfate esters¹¹ and produce a mixture of halodiols (7). The diols were further functionalized with a 2-naphthoate ester on the primary hydroxyl group, affording 8 with a chromophore for HPLC purification.

Owing to the hydrophobicity of the mono-2-naphthoate esters, initial attempts at HPLC purification employed normal phase separation conditions; however, this approach failed to achieve adequate separation. After screening various reversed phase HPLC conditions, we found that a semipreparative C₁₈ chromatography column in conjunction with an isocratic acetonitrile/H₂O method delivered separation at what appeared to be an acceptable resolution. However, analysis of the fractions containing the most nonpolar constituent in the mixture revealed a roughly 1:1 mixture of the desired hexabrominated lipid 9 and (apparently a single isomer of) a pentabromo variant. Further evaluation of HPLC conditions allowed for a clean separation of the two compounds when an analytical column possessing a phenyl-hexyl capped silanol stationary phase was employed. Milligram quantities of the naphthoate ester derivative of the hexabrominated lipid (9) were isolated in this way, beginning from cultures of ca. 5 L. Exploratory studies indicated that the direct use of the latter HPLC conditions would afford pure hexabromide 9 from mixture 8; however, we were not in possession of a preparative version of the phenyl-hexyl capped silanol stationary phase column.

Initial comparison of the ¹H NMR spectra of compound 9 and the diol derivative of danicalipin A⁴ revealed a remarkably similar set of resonances attributed to the stereochemically complex portion of the two compounds. These data were the first suggestion that not only did the halogenase machinery of *O. danica* install bromides with the exact same constitutional and stereochemical pattern as it did with chlorides, but the corresponding hexabromide diol also likely adopted the same solution conformation as the hexachloride. These initial tentative conclusions were corroborated by application of Murata's J-based configurational analysis method^{12–14} on compound 9,¹⁵ and of course by the Carreira group's recent synthetic campaign. Finally, 2-naphthoate ester 9 was converted to diol 11 by treatment with DIBAL-H, converging on the synthetic compound just reported by Carreira and co-workers,⁹ providing further independent support for our structural assignment.

In short, we have carefully evaluated the statement made by Haines over 40 years ago that the alga *O. danica*, known as a producer of chlorosulfolipids, could also generate bromosulfolipids under bromide-enriched growth conditions. Moreover, we have painstakingly isolated a derivative of the most highly brominated compound from the crude mixture of halosulfolipids produced by the organism and shown that the halogenation pattern is identical to that of the chlorosulfolipid danicalipin A, the most highly chlorinated lipid generated under "normal" growth conditions.

■ ASSOCIATED CONTENT

§ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00230.

Experimental protocols for growth of *Ochromonas danica* in bromide-containing media; isolation, synthetic manipulations, purification, and characterization of compounds 9 and 11 (PDF)

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The authors declare no competing financial interest.

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