Title
The Alga Ochromonas danica Produces Bromosulfolipids

Journal
Organic Letters, 18(5)

ISSN
1523-7060

Authors
White, Alexander R
Duggan, Brendan M
Tsai, Shiou-Chuan
et al.

Publication Date
2016-03-04

DOI
10.1021/acs.orglett.6b00230

Peer reviewed

HHS Public Access

Author manuscript

Org Lett. Author manuscript; available in PMC 2018 June 27.

Published in final edited form as:

Org Lett. 2016 March 04; 18(5): 1124–1127. doi:10.1021/acs.orglett.6b00230.

The Alga *Ochromonas danica* Produces Bromosulfolipids

Alexander R. White[a], **Brendan M. Duggan**[b], **Shiou-Chuan Tsai**[*,a,c], and **Christopher D. Vanderwal**[*,a]

[a]1102 Natural Sciences II, Department of Chemistry, University of California, Irvine, CA 92617

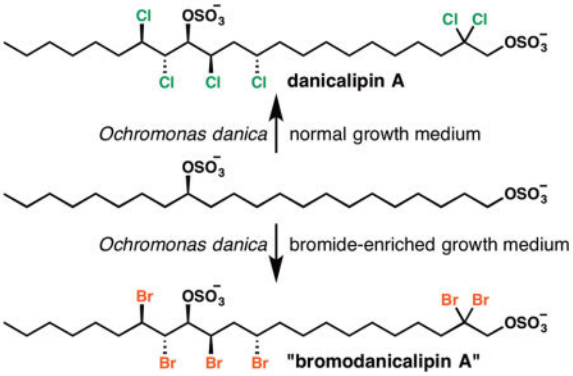
[b]9500 Gilman Drive, #0684, School of Pharmacy & Pharmaceutical Science, University of California, San Diego, CA 92093

[c]3205 McGaugh Hall, Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92617

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.

Graphical Abstract



The chlorosulfolipids (Figure 1) are fascinating halogenated lipids that were first isolated in the late 1960s from the fresh-water 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

[*]Corresponding Author: sctsai@uci.edu, cdv@uci.edu.

Supporting Information

Experimental protocols for growth of *Ochromonas danica* in bromide-containing media; and isolation, synthetic manipulations, purification, and characterization of compounds **9** and **11** (PDF). The Supporting Information is available free of charge on the ACS Publications website.

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 bromine[8]—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 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 of 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 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_5Cl, 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.

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 rendered the isolation of our target bromosulfopid 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 isolation[5] 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 esters[11] 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 reverse phase HPLC conditions, we found that a semi-preparative C_{18} chromatography column in conjunction with an isocratic acetonitrile/H_2O 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 evalution 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 ^{1}H NMR spectra of compound **9** and the diol derivative of danicalipin A[4] 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge the expert assistance of Drs. John Greaves and Beniam Berhane of the UCI Mass Spectrometry Facility. We thank Prof. William Gerwick (UCSD and SIO) for establishing our collaboration between the UCI group and Dr. Brendan Duggan. Early parts of this work were supported by the NIH through grant GM-086483.

References

1. For reviews of the chlorosulfolipids, see: Haines TH. Prog Chem Fats Lipids. 1971; 11:297–345.Haines TH. Annu Rev Microbiol. 1973; 27:403–412. [PubMed: 4593937] Haines TH. Lipids and Biomembranes of Eukaryotic Microorganisms. Erwin JA. Academic PressNew York1973:197–232.Bedke DK, Vanderwal CD. Nat Prod Rep. 2011; 28:15–25. [PubMed: 21125121] Kawahara T, Okino T. Studies in Natural Products Chemistry. 2012; 36:219–247.Nilewski C, Carreira EM. Eur J Org Chem. 2012; 9:1685–1698.Chung, W-j, Vanderwal, CD. Acc Chem Res. 2014; 2:718–728.Umezawa T, Matsuda F. Tetrahedron Lett. 2014; 55:3003–3012.
2. Elovson J, Vagelos PR. Proc Natl Acad Soc USA. 1969; 62:957–963.(b) Elovson J, Vagelos PR. Biochemistry. 1970; 9:3110–3126. [PubMed: 4321366]
3. (a) Haines TH, Block RJ. J Protozool. 1962; 9:33–38.(b) Mayers GL, Haines TH. Biochemistry. 1967; 6:1665–1671. [PubMed: 6035907] (c) Mayers GL, Pousada M, Haines TH. Biochemistry. 1969; 8:2981–2986. [PubMed: 4309129] (d) Haines TH, Pousada M, Stern B, Mayers GL. Biochem J. 1969; 113:565–566. [PubMed: 5812095]

4. Bedke DK, Shibuya GM, Pereira A, Gerwick WH, Haines TH, Vanderwal CD. J Am Chem Soc. 2009; 131:7570–7572. [PubMed: 19445461]
5. Kawahara T, Kumaki Y, Kamada T, Ishii T, Okino T. J Org Chem. 2009; 74:6016–6024. [PubMed: 19627129]
6. For chemical syntheses of danicalipin A, see: (a) ref 4. Yoshimitsu T, Nakatani R, Kobayashi A, Tanaka T. Org Lett. 2011; 13:908–911. [PubMed: 21288044] Umezawa T, Shibata M, Kaneko K, Okino T, Matsuda F. Org Lett. 2011; 13:904–907. [PubMed: 21291262] Chung, W-j, Carlson, JS., Vanderwal, CD. J Org Chem. 2014; 79:2226–2241. [PubMed: 24494597] Bailey AM, Wolfrum S, Carreira EM. Angew Chem Int Ed. 2016; 55:639–643.
7. In a search of Web of Science, we have found records of two meeting abstracts on the subject. The first is entitled “Halosulfolipids of Ochromonasdanica”: Haines TH, Pousada M, Bruckstein A, Das B. Journal of the American Oil Chemists Society. 1971; 48:A98., for which the abstract can be obtained. The second is entitled “Bromosulfolipids of Ochromonasdanica”: Pousada M, Bruckstein A, Haines TH, Das BC. Abstracts of Papers of the American Chemical Society. 1971:156.
8. For a recent, highly relevant example, see: Zhu Q, Hillwig ML, Doi Y, Liu X. ChemBioChem. published online 8 January 2016.
9. Fischer S, Huwyler N, Wolfrum S, Carreira EM. Angew Chem Int Ed. 2016; 55:2555–2558.
10. Foster BLL, Chrzanowski TH. J Microbiol Methods. 2012; 88:322–324. [PubMed: 22230234]
11. Mooney CL, Mahoney EM, Pousada M, Haines TH. Biochemistry. 1972; 11:4839–4844. [PubMed: 4347704]
12. Matsumori N, Kaneno D, Murata M, Nakamura H, Tachibana K. J Org Chem. 1999; 64:866–876. [PubMed: 11674159]
13. For the use of the Murata method for the determination of chlorosulfolipid configurations, see: Ciminiello P, Fattorusso E, Forino M, Di Rosa M, Ianaro A, Poletti R. J Org Chem. 2001; 66:578–582. [PubMed: 11429832] Ciminiello P, Dell’Aversano C, Fattorusso E, Forino M, Magno S, Di Rosa M, Ianaro A, Poletti R. J Am Chem Soc. 2002; 124:13114–13120. [PubMed: 12405839] Ciminiello P, Dell’Aversano C, Fattorusso E, Forino M, Magno S, Di Meglio P, Ianaro A, Poletti R. Tetrahedron. 2004; 60:7093–7098.(d) ref 4. Pereira AR, Byrum T, Shibuya GM, Vanderwal CD, Gerwick WH. J Nat Prod. 2010; 73:279–283. [PubMed: 20099812]
14. After it had originally been directly adapted to address the chlorosulfolipids, Carreira and co-workers refined the Murata method for application to polychlorinated molecules: Nilewski C, Geisser RW, Ebert M-O, Carreira EM. J Am Chem Soc. 2009; 131:15866–15876. [PubMed: 19817365]
15. We have found in previous work on danicalipin A that the preferred low-energy solution conformation of the C11–C16 segment remains the same in the both sulfated and desulfated forms. Okino and Carreira have observed the same phenomenon; see refs 5 and 9.

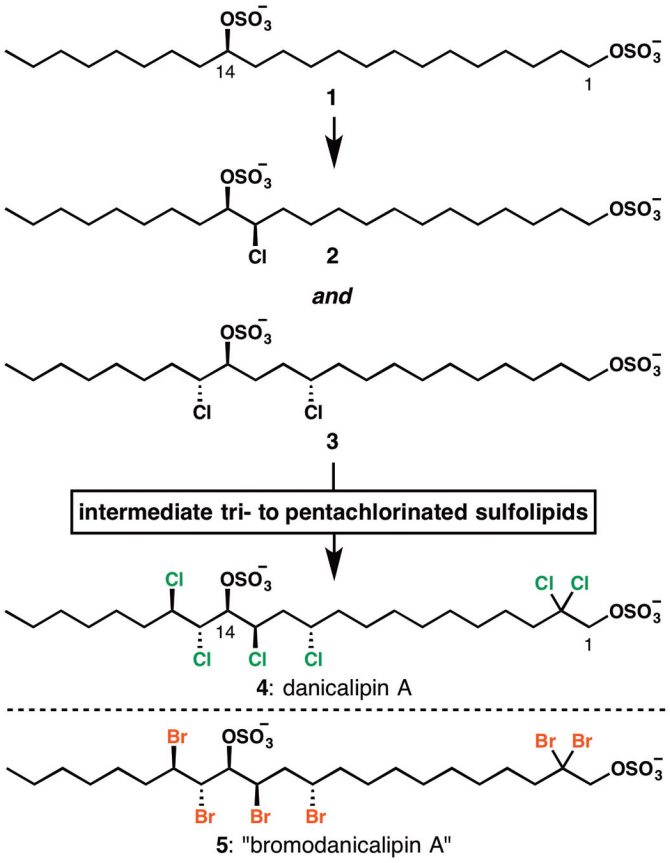


Figure 1.
Currently understood sequence for chlorination in the biosynthesis of danicalipin A

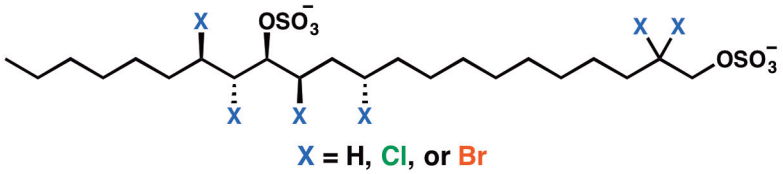
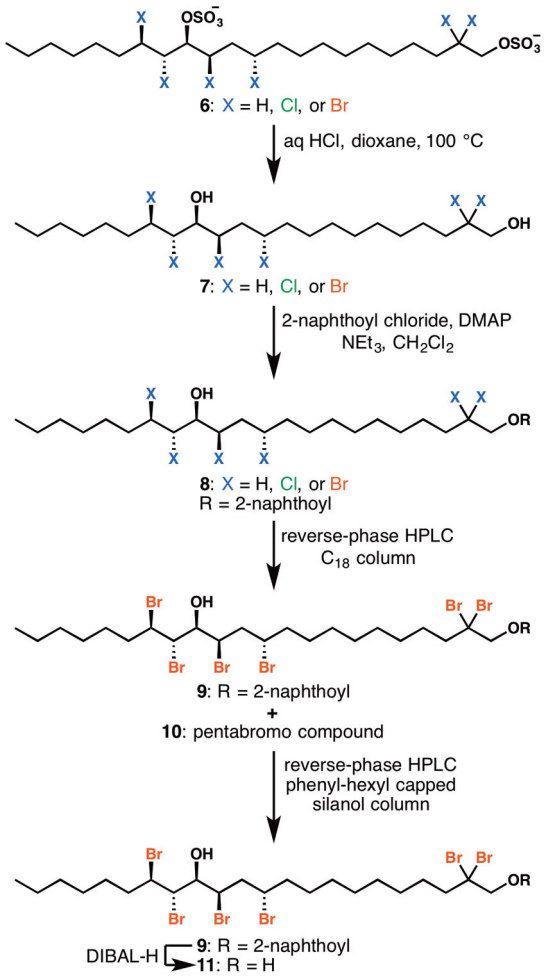


Figure 2.
The potential permutations of halosulfolipids produced in bromine-enriched medium



Scheme 1.

Production and isolation of bromodanicalipin A diol (**11**)