

Revisiting *Fascaplysinopsis reticulata* and the evaluation of ASE versus Kupchen type extraction

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ABSTRACT

Fascaplysinopsis reticulata has been studied for nearly twenty years and found to contain the major metabolite fascaplysin as well as many other analogs. The main goal of this project was to reinvestigate the chemistry of a new sample of *F. reticulata* collected from Papua New Guinea (PNG). A 2002 collection, 02161, contained fascaplysin (**2**), homofascaplysin A (**3**) and reticulatine (**5**). A 2005 collection, 05417, contained homofascaplysate A (**1**) and reticulatol (**4**) in addition to compounds **2**, **3** and **5**. Also noteworthy was that compound (**1**) appeared to form a salt with bromine versus the previously observed chlorine anion. Another portion of this research evaluated the performance capabilities of accelerated solvent extraction (ASE) versus a modified Kupchen-type extraction. There have been speculations that the ASE extraction method degrades compounds because high pressure and temperature are the crucial components of this method. In analyzing the dichloromethane fractions from the ASE and Kupchen-type extractions it was discovered that the ASE yielded a cleaner extract, although in lower yields: 230 mg versus 395.8 mg, but in a rapid fashion: 3 hours versus 120 hours and with no apparent degradation of chemistry.

INTRODUCTION

Importance of *Fascaplysinopsis reticulata*

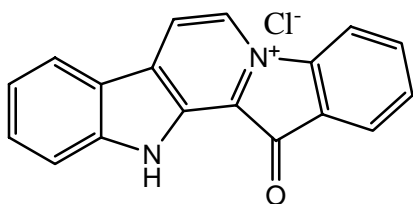
The structure of fascaplysin was first reported by the Ireland group of the University of Utah in collaboration with the Clardy group of Cornell University in 1988.² After fascaplysin was discovered it was soon determined that it had worthwhile biological activity; therefore, it was important to take a second look at a recent collection of *Fascaplysinopsis reticulata* from Papua New Guinea (PNG). It was previously discovered in 2004 that an *F. reticulata* sponge collected in PNG in 2002 contained fascaplysin (**2**) and two derivatives: homofascaplysin A (**3**) and reticulatine (**5**).¹ One of the main goals of this research was to discover if an *F. reticulata* sponge collected in a different area of PNG several years later would have different chemistry.

Fascaplysin has had an interesting journey. The Ireland and Clardy group discovered that fascaplysin inhibited the growth of several microbes and suppressed proliferation of the leukemia cell line L-1210 with an ED₅₀ of 0.2 µg/mL.² In 2000 Novartis reported that in an *in vitro* study fascaplysin selectively inhibited Cdk4 and in 2006 the Jenkins' group reported that the IC₅₀ for Cdk4 inhibition was 0.55 µM.^{3,4} In 2001 Novartis also

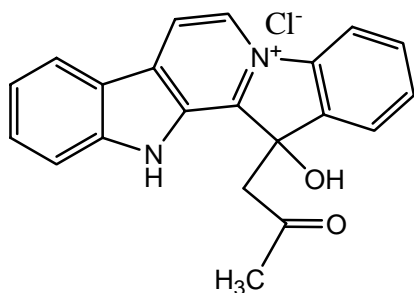
reported that faspaplysin is a DNA intercalator.⁵

Dr. Fred Valeriote at the Henry Ford Health System reported in 2005 that faspaplysin showed selectivity in *in vitro* solid tumor selective assays. It was discovered that the preclinical dose was a maximum injectable dose of 100 µg/mouse, which was not toxic for the mice; however, they reported that a maximum tolerated dose (MTD) could not be achieved, so the maximum injectable dose was used. Valeriote reported that pharmacokinetics showed that faspaplysin would have therapeutic efficacy as a chronic drug treatment; however, it proved to be too toxic for a bolus treatment.⁶

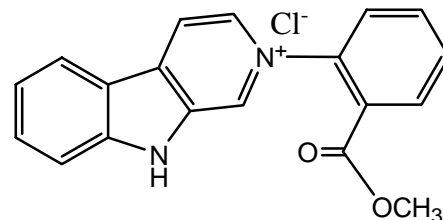
Dr. Seagraves, who was once a graduate student in the Crews lab, isolated three compounds from a 2002 collection of an *F. reticulata* sponge from PNG. The compounds he isolated from sponge 02161 were: faspaplysin (**2**), homofaspaplysin A (**3**) and reticulatine (**5**).



2



3



5

One of the goals of this research was to determine if the same species of sponge would produce different compounds in a slightly different geographical area and in a span of three years.

ASE versus Kupchen-Type Extraction

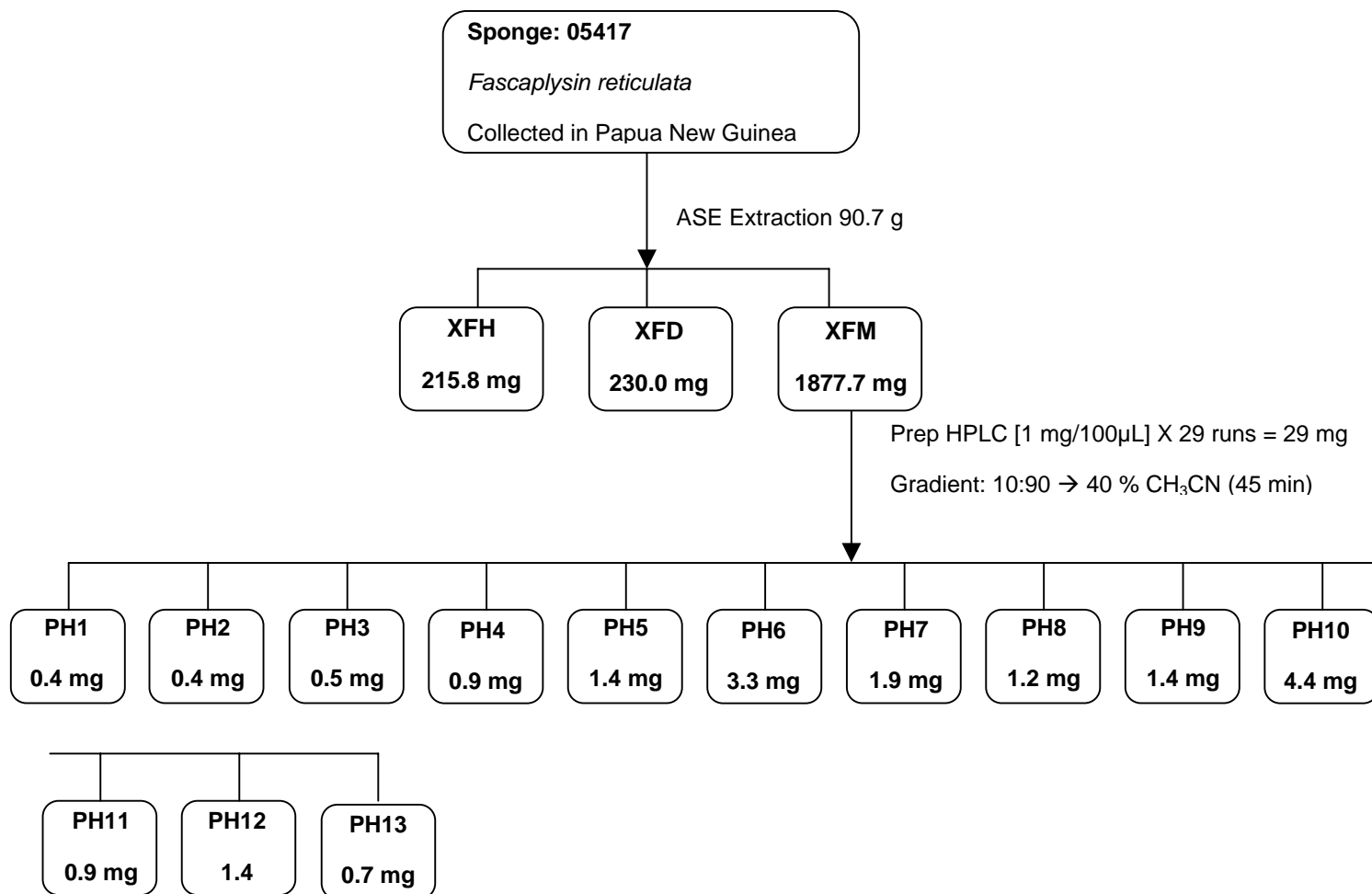
Another caveat to this experiment was analyzing the benefits of using accelerated solvent extraction (ASE) over a modified Kupchen extraction, or *visa versa*. No papers have been published on the effects of ASE versus the Kupchen-type extraction method that most natural product structure elucidation laboratories use. Soxhlet extraction has been compared to the ASE method a few times in the literature, but our group is interested in knowing whether or not the ASE method would be the most productive and not cause any degradation to the compounds being isolated.^{7,8} The modified Kupchen extraction method is very gentle to compounds. The concern is that since the ASE method uses high pressure and temperature to extract compounds from sponges those compounds being isolated may degrade due to the harsh extraction conditions.

Speed of extraction is also a variable because it takes roughly five days to extract a sponge using the modified Kupchen extraction method; however, in an hour and a half a sponge can be extracted using the ASE extraction method.

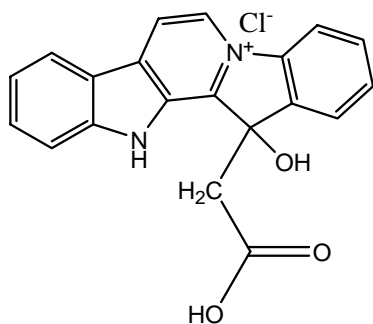
RESULTS AND DISCUSSION

For the first portion of this research the goal was to determine whether the same species of sponge collected in slightly different areas of PNG with a span of three years between their collections would have the same or different chemistry. Sponge 05417 was extracted using the ASE method. LCMS was used to guide further purification after ASE extraction and to determine the proper gradient for further separation on an HPLC. From the LCMS data it was determined that the XFM, methanol, extract contained the most chemistry; therefore, that fraction was further purified using reverse-phase preparatory HPLC. The isolation scheme of 05417 is shown below.

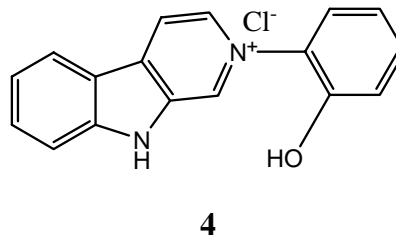
Scheme 1. Isolation scheme of *F. reticulata* sponge to yield pure compounds.



Through the use of MS it was determined that 05417 contained fascaplysin and four analogs: PH5 – homofascaplysin (1), PH6 – 2, PH7 – 3, PH9 – reticulatol (4), PH10 – 5.



Compound (1)



4

It was thought early on in this research that compound (1) might be novel; however, it matched the literature NMR values of homofascaplysin A fairly closely. All carbon peaks of compound (1) matched the literature values except that they were shifted approximately 1.4 ppm. All the proton peaks were very close to the

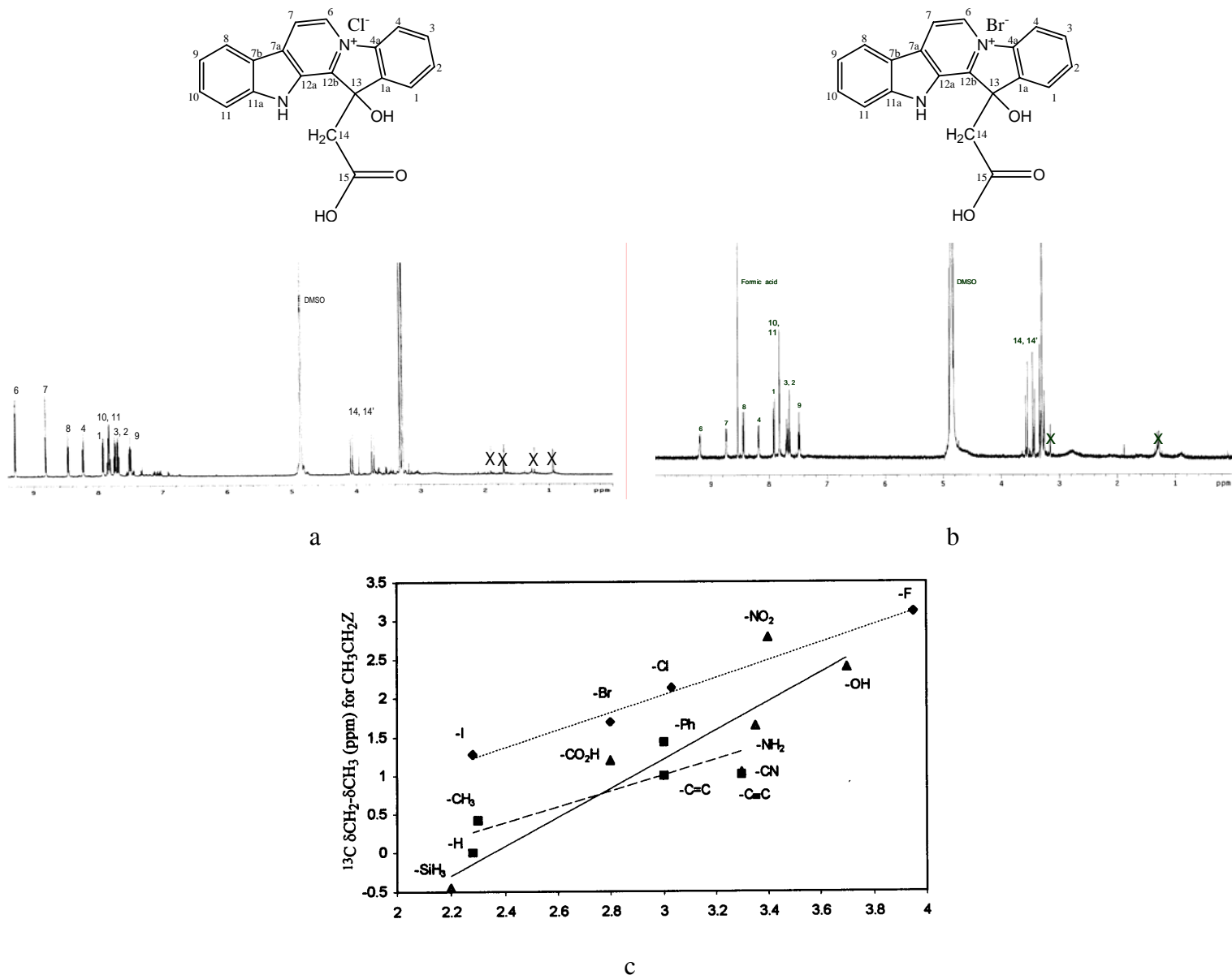
literature values except that the methylene protons in compound (**1**) were closer together and shifted upfield compared to that of homofascaplysate A, Figure 1. It is speculated that a less electronegative halogen than chlorine, such as bromine, may be ionically bound to compound (**1**) because that could cause such a shift. Since compound (**1**) has only one stereo center it cannot be a diastereomer of homofascaplysate A, and compound (**1**) cannot be an enantiomer of homofascaplysate A because then all the NMR values would match exactly. The NMR values and literature values of compound (**1**) are shown in Table 1. The structures of compounds **2**, **3**, **4**, and **5** were elucidated through the use of an MS and NMR comparison to literature values .

Table 1. NMR data for Homofascaplysate A (**1**).

Position	δ_{C}	Literature δ_{C}	δ_{H} (<i>J</i> in Hz)	Literature δ_{H} (<i>J</i> in Hz)
1	124.589	126.0	7.920 d (1.5, 7.5)	7.93 ddd (0.6, 1.4, 7.5)
1a	138.418	139.8		
2	130.172	131.6	7.651 ddd (0.5, 8, 15.5)	7.69 ddd (1.0, 7.5, 7.5)
3	130.316	131.7	7.700 ddd (1.5, 7.5, 15.0)	7.74 ddd (1.3, 7.8, 7.8)
4	113.665	115.1	8.185 dd (8.0)	8.23 d (8.0)
4a	140.888	142.3		
6	122.793	124.2	9.201 d (6.5)	9.28 d (6.6)
7	116.649	118.1	8.743 d (6.5)	8.81 d (6.5)
7a	134.969	136.4		
7b	120.066	121.5		
8	123.001	124.5	8.447 d (8.0)	8.47 ddd (1.0, 1.0, 8.0)
9	121.910	123.4	7.489 ddd (1.0, 3.5, 8.0)	7.51 ddd (1.5, 6.5, 8.1)
10	132.209	133.6	7.825 dd (2, 3)	7.84 m
11	112.702	114.1	7.825 dd (2, 3)	7.84 m
11a	145.236	146.6		
12a	131.086	132.4		
12b	144.642	146.0		
13	79.271	80.7		
14,14'	44.187	45.3	3.565 d (16.0), 3.444 d (16.0)	4.07 d (17.0), 3.74 d (17.0)
15	174.496	176.0		

Data was obtained on a 500 MHz MNR in MeOH-*d*₄

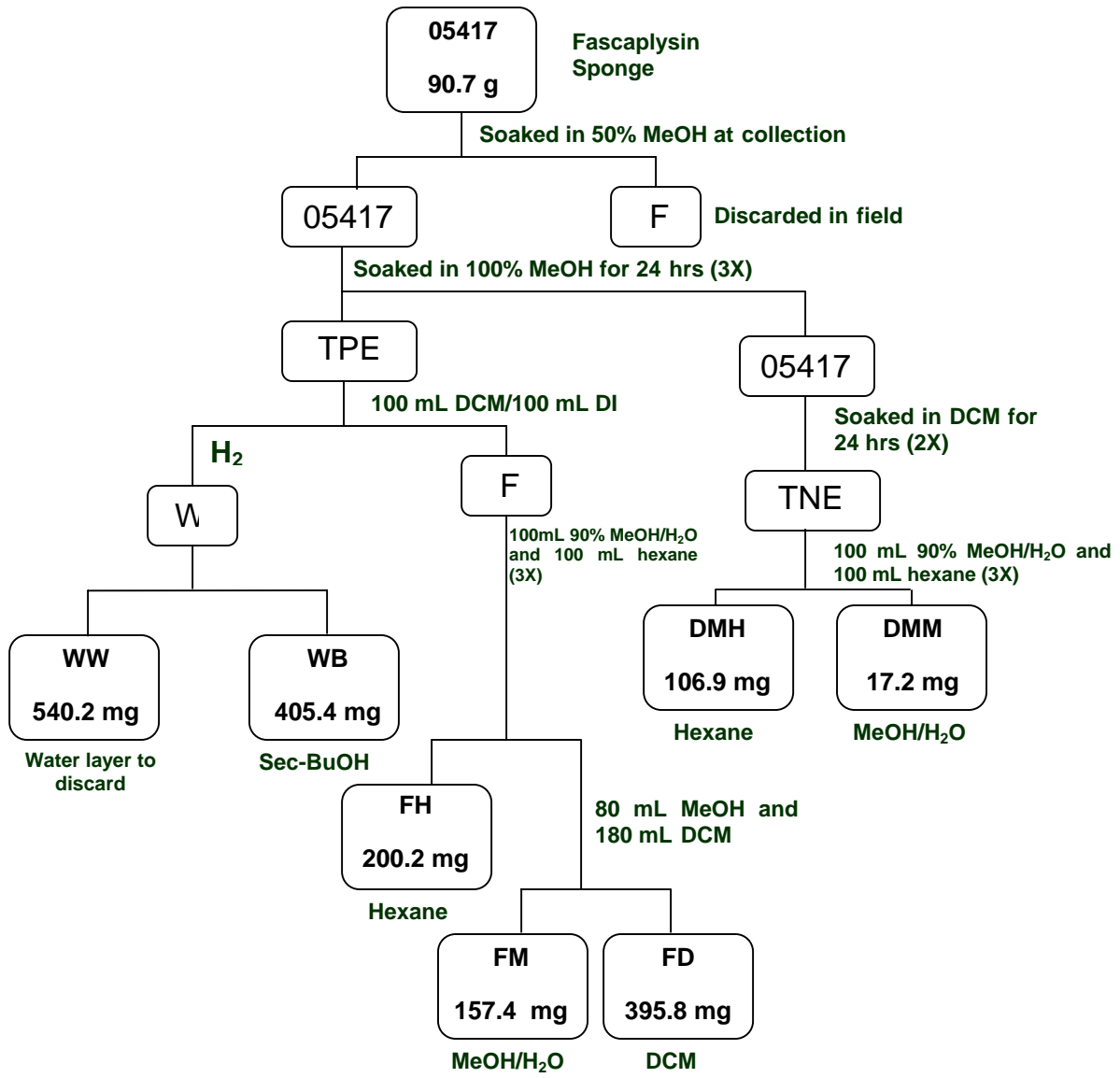
Figure 1. Putative binding of halogens to homofascaplysate A: a) Cl^- , b) Br^- , c) effects of electronegativity in ^1H chemical shift.¹¹



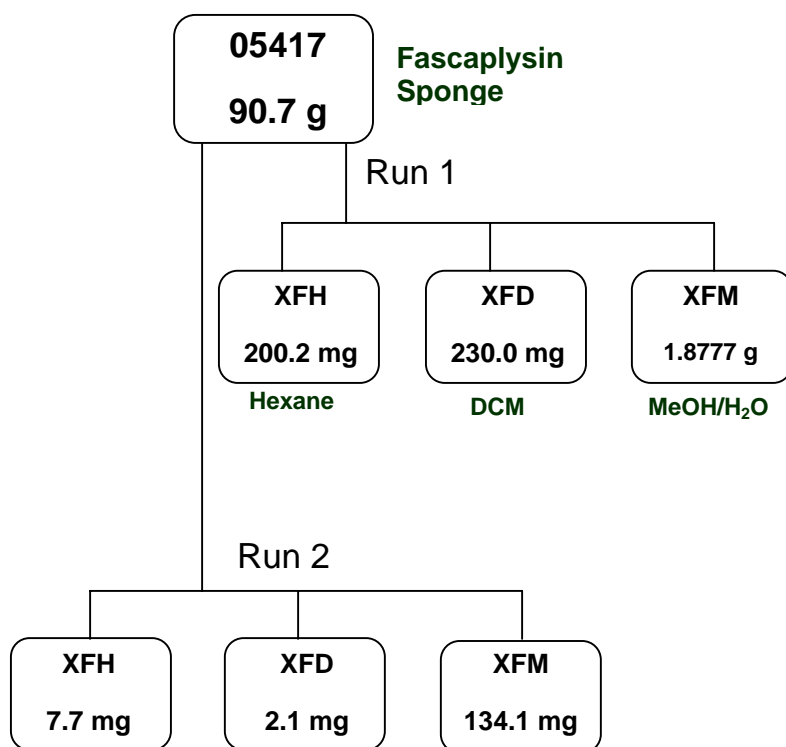
A silver nitrate test was performed to detect what halogen is bound to compound (**1**); however, this test did not yield reliable results. An MS in negative ion mode was also obtained, but no halogen could be detected. Further experimentation is underway to determine what halogen is bound compound (**1**).

For the second portion of this research 90.7 g of sponge 05417 was extracted using the Kupchen-type extraction and 90.7 g was extracted using the ASE extraction method. Both extraction schemes are shown below.

Scheme 2. Modified Kupchen extraction scheme of *F. reticulata* sponge 05417.



Scheme 3. ASE extraction scheme of *F. reticulata* sponge 05417.



By analyzing the LCMS trace of the crude FM fraction from the Kupchen-type extraction and comparing that to the LCMS trace of the XFM fraction from the ASE extraction it was determined that the ASE actually yielded a cleaner fractionation. It was also found that the MS trace of the XFM fraction contained four different masses that that FM trace did not. This leads one to speculate that the ASE may be performing a more thorough isolation than the Kupchen-type extraction. An NMR of the crude FM and XFM fractions was also taken and it confirmed what was seen in the LCMS trace, that the ASE yielded a cleaner extraction.

CONCLUSIONS

It was concluded in the first portion of this experiment that compound (**1**) is indeed homofascaplysate A; however, further experimentation would need to be performed to determine exactly what halogen is in solution with compound (**1**). The most exciting result from this research is that one species of sponge collected in 2002 in a slightly different geographical location than the exact same species collected in 2005 produced different chemistry. It was found that the 2005 sponge contained two additional fascaplysin analogs: **1** and **4**, which were not observed in the 2002 sponge.

There is still much experimentation required to propose an accurate conclusion for the second portion of this research. However, from the data obtained thus far it can be speculated that the ASE extraction is not degrading compounds and is a more time efficient method of extraction. Future research will entail performing both methods of extraction on three other species of sponges with varying chemistry: *Cacospongia mycofijiensis*, *Jaspis coriacea*, and *Auletta cf. constricta*. The main metabolites from each sponge will be isolated using both extraction methods. The yields, LCMS and NMR data of the crudes and the main metabolites will be compared.

EXPERIMENTAL SECTION

General Experimental Procedures

All 1-D NMR data was obtained on a 500 MHz Varian NMR in MeOH-*d*₄. NMR assignments were based on literature values.¹ ESIMS data was obtained on an Applied Biosystems Mariner. An LCMS of the crude extract was obtained by using a 5 μm analytical column with a photodiode array and evaporative light scattering detector. A reverse-phase preparative HPLC was used to separate the crude fraction with a UV detector set to a wavelength of 230 nm.

Extraction and Isolation

The *Fascaplysinopsis reticulata* sponge (UCSC collection 05417) was stored in MeOH since it was abstracted from its origin site in PNG. The sponge was then extracted using a DIONEX ASE (Accelerated Solvent Extractor) 100 by three solvents: hexane, dichloromethane, and methanol. The methanol extract, labeled “FM,” was then further purified using a reverse-phase preparative-HPLC to yield the pure compounds. The HPLC gradient used was 10:90 to 40:60 ACN-H₂O (0.1% formic acid was added to each solvent) over 45 minutes.

ACKNOWLEDGMENT

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