

Pyrroloacridine Alkaloids from *Plakortis*

quasiamphiaster

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ABSTRACT

In an attempt to isolate more of the unpublished Plakinidine E (3) from the marine sponge *Plakortis quasiampfiaster*, a set of possible novel compounds were discovered. A compound having m/z 289 was isolated from the sponge using reverse-phase HPLC. Comparison of literature values and NMR experiments lead to the conclusion that the m/z 289 was Plakinidine D (2) with Plakinidine A (1) as an impurity. Plakinidine D has only been previously isolated from a sea squirt. A compound of m/z 322, which matches no known plakinidines from the sponge, was also isolated.

INTRODUCTION

Marine organisms are good sources of novel compounds. These organisms produce secondary metabolites, which are not essential for life. However these chemicals may be produced as a means of chemical defense since many marine invertebrates are all sessile, soft bodied, and filter feeders. The secondary metabolites created in response to these properties have an interesting host of biological properties. Among these slew of compounds, alkaloids constitute the largest group.¹ More specifically, pyridoacridines and pyrroloacridines are of particular interest because they have a variety of interesting biological activities. These compounds have the ability to intercalate DNA and have telomerase and topoisomerase II inhibitory activities.¹ These abilities are specifically important in inhibiting the growth of cancerous cells, making these compounds ideal for developing novel anticancer drugs. The main difference between these two groups of compounds is whether a pyridine or pyrrole is joined to the acridine core (Figure 1).¹ This paper will focus on pyrroloacridines because they are far less common than pyridoacridines.

To date, the only pyrroloacridines that have been published from marine sources are plakinidines A-D and alpinkidine. Plakinidine A-C were discovered by two separate research groups from *Plakortis* spp.^{2,3} Plakinidine A and B demonstrated in vitro activity against the parasite *Nippostrongylus brasiliensis*,³ and plakinidine A also demonstrated activity against HCT-116 and H-125 M in a Josephine Ford Cancer Center bioactivity study. Plakinidine D is different in that it has only been isolated from *Didemnum* sp., which is not a sponge, but a sea squirt. Since plakinidine D was isolated from a different species of marine organism than A-C, there is a question of whether the sponge is producing these secondary metabolites or if it is being produced by some other microorganism.⁴ Plakinidine D also showed in vitro cytotoxicity against HCT-116.⁴

This paper focuses on *Plakortis quasiampfiaster* (03404 UCSC collection) and the isolation of compounds from this sponge. Amphiasterins which are mildly cytotoxic have also been isolated.⁵ They have a very different structure than the plakinidines, consisting of γ -lactones with greasy side chains. Not much work has been done with the *Plakortis quasiampfiaster* because Plakinidine A and B and the

amphiasterins are the major components in the sponge and they tend to bleed into the other minor compounds during chromatography. Previously, a compound, which has been dubbed plakinidine E (3), was isolated from this 03404 collection. NMR experiments showed that this compound is a pyrroloacridine alkaloid but with a different molecular formula by mass spectrometry. High-resolution mass spectrometry gave a molecular formula of $C_{17}H_{12}N_3O_2$ for plakinidine E and the structure was determined by a combination of 1D and 2D NMR experiments. Unfortunately plakinidine E degraded shortly after. The aim of this research is isolate more plakinidine E for further NMR and bioactivity studies.

EXPERIMENTAL PROCEDURE

The sponge was previously extracted using Accelerated Solvent Extraction. All reverse phase-HPLC was done using a gradient of 10-100% ACN/H₂O in 1.25% formic acid on a 25mm column. NMR data was measured in *dmso-d₆* at 500MHz (¹H) and 125 MHz (¹³C). Mass spectrometry data was collected using a Mariner time-of-flight electrospray ionization mass spectrometer.

RESULTS AND DISCUSSION

In order to isolate more 3, the crude methanol soluble extract of the sponge was dissolved in methanol and ran on the prep- HPLC (Scheme 1). The separation yielded 12 fractions with fraction P8 containing *m/z* 290. There was not much collected from this fraction, so all the fractions previously collected from the original project that contained any amount of *m/z* 290 were gathered together and purified. P8 along with these fractions were purified only to discover that *m/z* 290 had for the most part disappeared. There were traces of *m/z* 290 but it was always with 1 as the major or equal component in the NMR experiments, and there would not have been enough to purify further. At this point since *m/z* 290 was mostly gone, the MS data was inspected to show that there was *m/z* 289 instead of or with *m/z* 290. Therefore, it was hypothesized 3 was not ionizing properly when it was being separated in the HPLC. A slower gradient would be required to try to better separate the major and the minor components.

Using a slower gradient the crude methanol soluble extract was run through the prep-HPLC again. This time 11 fractions were collected with a new peak (Figure 3) coming out at 34% ACN. MS was run on all the fractions to find that P5 had m/z 322 and P11 had m/z 289. High resolution MS revealed the possible formulas for m/z 322 to be $C_{17}H_{12}N_3O_4$ which is not similar to any of compounds previously isolated from *Plakortis quasiampfiaster*. Due to time constraints the focus of the research shifted onto the characterization of m/z 289 and time permitting, the structural elucidation of m/z 322.

NMR experiments were conducted on fraction P11 because **2** had a molecular weight of 288 and it had never been collected from a sponge before. There was less than 1 mg of P11 collected so the nano probe was required to obtain ^{13}C NMR data. The chemical shifts of m/z 289 were compared to both **2** and **1** (Table 1). The carbon shifts match **2** with some discrepancies which can be contributed to the fact that **1** is also present as an impurity. 2D NMR was run on the sample and the shifts were correlated and calculated. The only major discrepancy between m/z 289 and **2** occurs at C2 in which m/z 289 has the protons shifted up field from the literature values. This could be due to the **1** in the sample.

One of the major ongoing complications of this project can be contributed to Plakinidine A and B, which are the major components of the sponge. Because there is such an abundance of these compounds they bleed into the minor components. One can see from Figure 3 that the largest peak in the chromatogram is **1** and it blends with the minor peaks. While the slower gradient seems to have separated m/z 322 from the major components an even slower gradient might be necessary to collect **2** from the sponge. In addition, the polarity of the plakinidines are so similar that they elute so closely by HPLC making the minor components difficult to purify. Another complication arises from the fact that the plakinidines are not soluble in normal NMR solvents. Deuterated dimethyl sulfoxide had to be used and is difficult to separate from the sample once it has been added. Commonly used NMR solvents (eg. Methanol and chloroform) requires a lot of acid to solubilize the compounds and can overwhelm these compounds in NMR experiments.

CONCLUSION

Plakinidine E could not be isolated from the sponge using a slow gradient on the prep-HPLC. An optimization of HPLC conditions is required for any future attempts to isolate 3. Plakinidine D, only previously isolated from a sea squirt, was successfully isolated from the *Plakortis quasiampfiaster* as a minor constituent. Also a possibly new compound was found in the attempt to isolate 3. The m/z 322 does not correlate to any known compounds from the sponge and the possible molecular formula is very similar to the plakinidines. NMR experiments are currently ongoing for this compound to try to elucidate the structure.

ACKNOWLEDGMENT

I would like to acknowledge Prof. Phillip Crews for allowing me to work in his lab. Also a huge thanks to Paul Ralifo and the other members of the Crews lab for helping me and teaching me useful skills. Special thanks to the National Science Foundation for funding my stay and giving me the opportunity to experience academic research.

APPENDIX

Figure 1. Representation of pyridoacridine versus pyrroloacridine

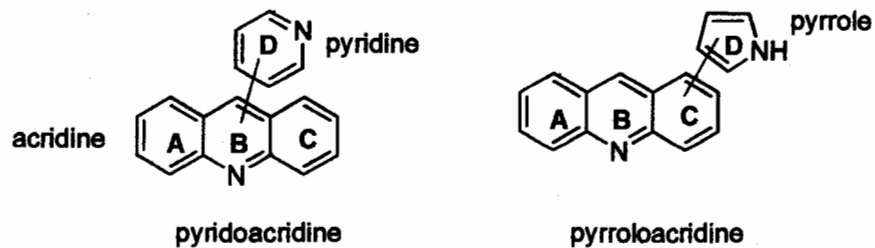
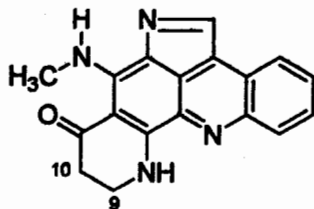


Figure 2. Proposed structure for Plakinidine E m/z 290 along with the structures of Plakinidine A and D.

plakinidine A (1)



plakinidine D (2)



plakinidine E (3)

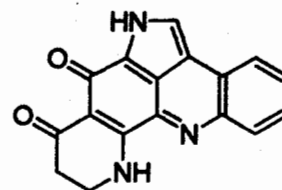
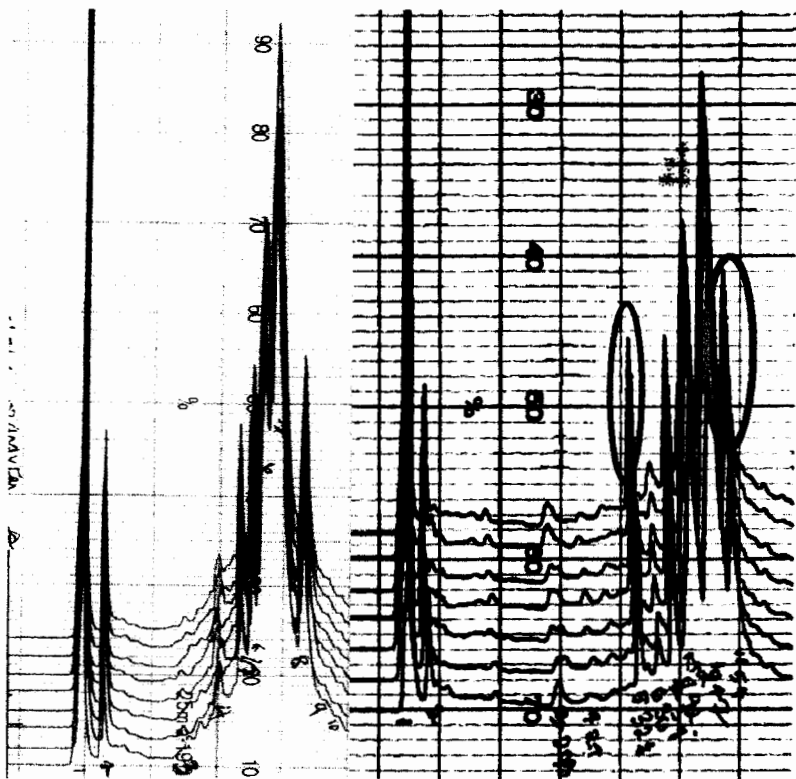


Figure 3. Original HPLC chromatograph versus the chromatograph from the slower gradient the red circle indicates the m/z 322 and the green circle indicates the m/z 289.



Scheme 1. Isolation scheme for Plakinidine E. Fractions containing m/z 290 are bolded.

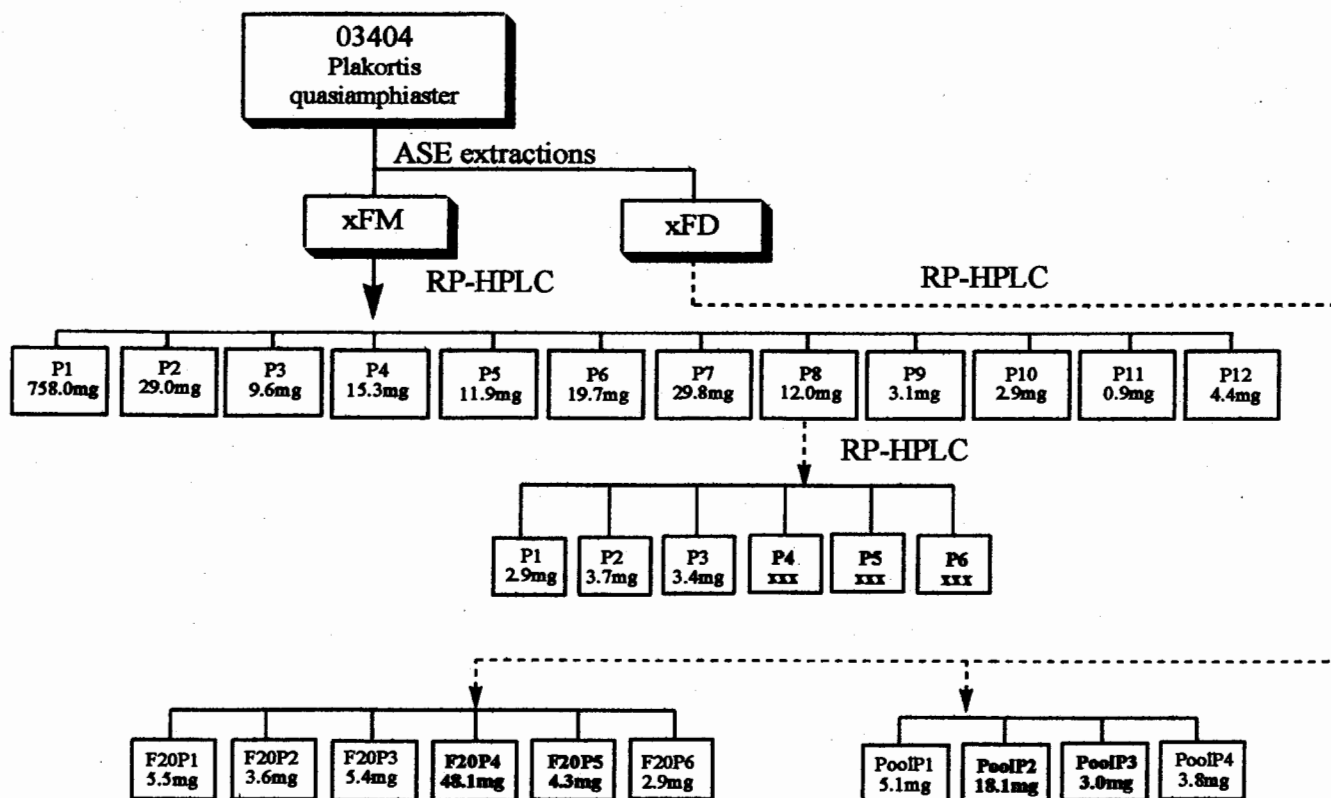


Table 1. Comparison of chemical shifts of unknown m/z 289 to plakinidines D and A.

C#	Plakinidine D ^a		03404xfmp11 m/z289		Plakinidine A ^b	
	δ_c	δ_H mult, J(Hz)	δ_c	δ_H mult, J(Hz)	δ_c	δ_H mult, J(Hz)
2	131	8.19s	131.41	7.65m	136.21	8.44s
2a	121.7		128.3		127.9	
2b	124.8		124.5		124.56	
3	124	8.25dd, 8.3, 1.1	123.76	8.36d, 7.8	123.94	8.38dd, 2.1, 6.7
4	129.3	7.81dt, 7.5, 1.1	126.79	7.67m	126.21	7.66m
5	127	7.77dt, 7.7, 1.2	127.8	7.72m	126.3	7.66m
6	130.8	8.31dd, 8.0, 1.1	130.47	8.23d, 7.8	130.44	8.24dd, 2.1, 6.9
6a	144.3		143.7		144.21	
7a	140		138.1		136.18	
7b	152		152.8		158.04	
8		8.36br s				9.85br s
9	40	4.05t, 7.2	36.44	3.81t, 7.8	36.93	3.81t, 7.7
10	35.9	2.89t, 7.2	35.64	2.72t, 7.8	25.65	2.71t, 7.5
11	193.8		194.22		194.15	
11a	99.6		100.1		100.08	
12	157.6		158		152.1	
12a	125.1		126.4		126.3	
12b	117.1		109		122.49	
13		10.9 br s				11.1q, 5.4
14			136.04	8.46	33.8	3.64d, 5.4
			94.3			
			60.56	4.24q, 7.2		
			26.9			
			34.06	3.67d, 4.8		

NMR Data is measured in DMSO-*d*₆ at 500 MHz (¹H) and 125 MHz (¹³C)

^aRef. 4.

^bRef. 2.

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