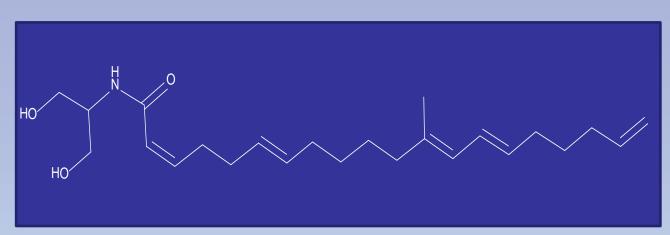


Marine Cyanobacterial secondary metabolites present important opportunities for pharmaceutical research. However, in order to effectively utilize these opportunities, taxonomic identification is necessary. Such identification is done through analysis of a ribosomal RNA unit known as 16s rRNA gene. This segment of the prokaryotic genome changes very little over time and thus allows for precise identification of very closely related organisms.

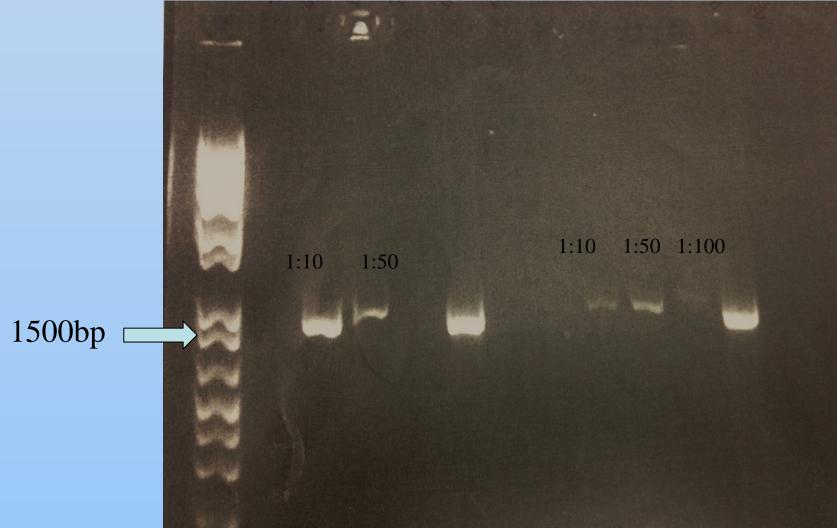
Useful Compounds of M.Bouillonii



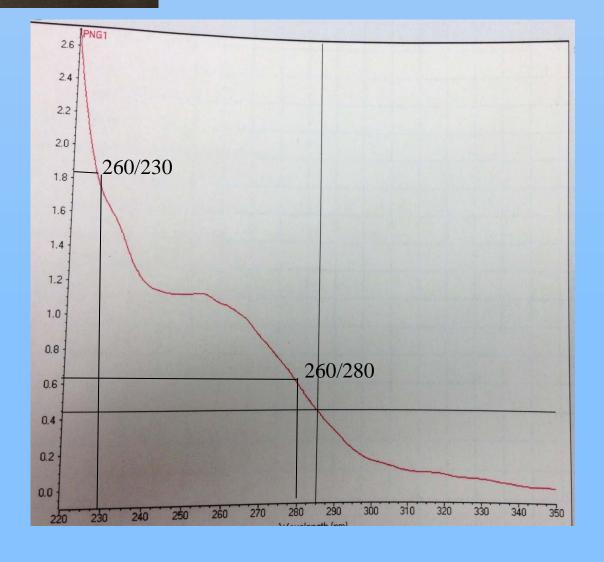
The amino-glycerol compound

The Gerwick chemistry lab isolated two compounds from the M.Bouillonii specimen; apratoxin A, which is known to be cytotoxic to pancreatic and lung cancer cells, and another previously unidentified amino glycerol compound. This second compound was found to have cannabinoid receptor modulatory effects, indicating its potential medical use as an appetite regulator, as well as a chemotherapy relief and anti-cancer agent.

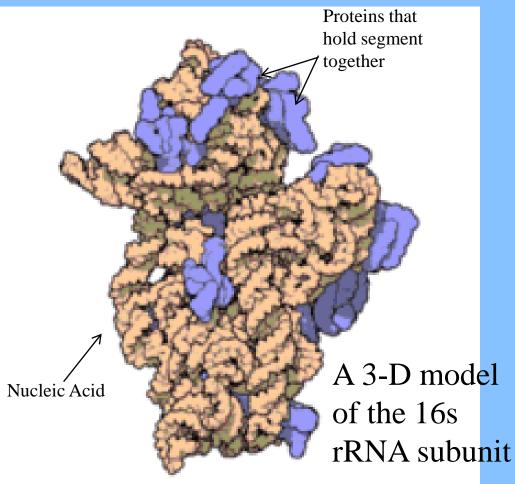
Testing the DNA: Nanodrop and Gel Electrophoresis



segment 10 cells.



This 1500 base pair sequence is used to identify the Cyanobacteria utilizing the very low rate of change from species to species. Only about 5 base pairs of this sequence are different related closely than other Cyanobacteria species¹. By identifying which base pairs are different, and the frequency of these alterations, a program can distinguish this organism from its relatives in a matter of seconds.



DNA Concentration: 53.1ng/ul 260/280: 1.81 260/230: 0.66

These Nanodrop results show a rather low concentration of DNA, however this is acceptable because large amounts of DNA are not needed in order to amplify the 16s rRNA gene. The 260/230 level, which indicates the level of DNA purity, is also very low, which is why the DNA dilutions were necessary. Impure DNA can result in PCR failure due to the other organic compounds that compromise the process. The 260/280 level is adequate indicating not too many impurities from proteins.

The 16s rRNA Sequence

GCTCGGGTCGATACAAGCTACACCTAGTATCCATCGTTTACAG CTAGGACTACTGGGGGTATCTAATCCCATTCGCTCCCC TAGCTTTCGTCCCTCAGTGTCAGGTTCAGTCCAGTAGAGCGC CTTCGCCACCGATGTTCTTCCCGAAATCTACGCATTC ACCGCTACACCGGGAATTCCCTCTACCCCTACTGCCCTCTAGT TCCTCAGTTTCCACTGCCTGCCCAGAGTTAAGCCCG GTCTTTAACAGCCGACTTGAGGTACCACCTACGGACGCTTTA CGCCCAATAATTCCGGATAACGCTTGCATCCTCCGTT TACCGCGGCTGCTGGCACGGAGTTAGCCGATGCTTTTCCTC AGGTACCGTCACTTTCTTCTTCCCTGATAAAAGAGGT TACAACCCAAAAGCCTTCCTCCCTCACGCGGTATTGCTCCGT CAGGCTTTCGCCCATTGCGGAAAATTCCCCACTGCGCCTCCC GTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGCTCA TCCTCTCAGACCAGCTACTGATCGTTGCCTTGGTAGGCTTTTA CCCTACCAACTAGCTAATCAGACGCGAGCTCCTCTTATGGCAA TAAATCTTTCACCTCTCGGCACATCCGGTATTAGCCACTGTTT CCGGTGGTTGTCCCCGACCCTAAGGCAGANTCTCACGCGTTA CTCACCCGTCCGCCACTCCCCCGAAGGGGGCGTTCGACTTGCA TGTGTTAAGCATACCGCCAGCGTTCATCCTGAGCCANGATCA AACTCTCACTGAGGGACGAAAGCTAGGGGAGCGAATGGGAT TAGATACCCCAGTAGTCCTAGCTGTAAACGATGGATACTAGGT GTAGCTTGTATCGACCCAAGCTGTGCCGAAGCCAACGCGTTA AGTATCCCGCCTGGGGGAGTACGCACGCAAGTGTGAAACTCA AAGGAATTGACGGGGGGCCCGCACAAGCGGTGGAGTATGTGG TTTAATTCGATGCAACGCGAAGAACCTTACCAGGGCTTGACA TGTCGCGAATCCCGGTGAAAGCTGGGAGTGCCTTAGGGGGC AGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGTTTT TAGTTGCCAGCACGTGAAGGTGGGCACTCTAGAGAGACTGC CGGTGACAAACCGGAGGAAGGTGGGGGATGACGTCAAGTCAG CATGCCCCTTACGCCCTGGGCGACACACGTACTACAATGGTC AGGACAATGGGCAGCCAACTCGCAAGGGGGGAGCTAATCTCA TCAAACCTGGCCTCAGTTCAGATTGCCGGCTGCAACTCGCCG GCATGAAGGAGGAATCGCTAGTAATCGCCGGTCAGAATACGG CGGTGAATCCGTTCCCGGGCCTTGTACACACCGCCCGTCACA CCATGGAAGCTGGCCACGCCCGAAGTCGTTACCCTAACCCTT TTGGGAGGGGGGGGGCCGAANGCAGGGTTGGTGACTGGGGGT GAAGTCGTAACAAGGTANCCGTAA

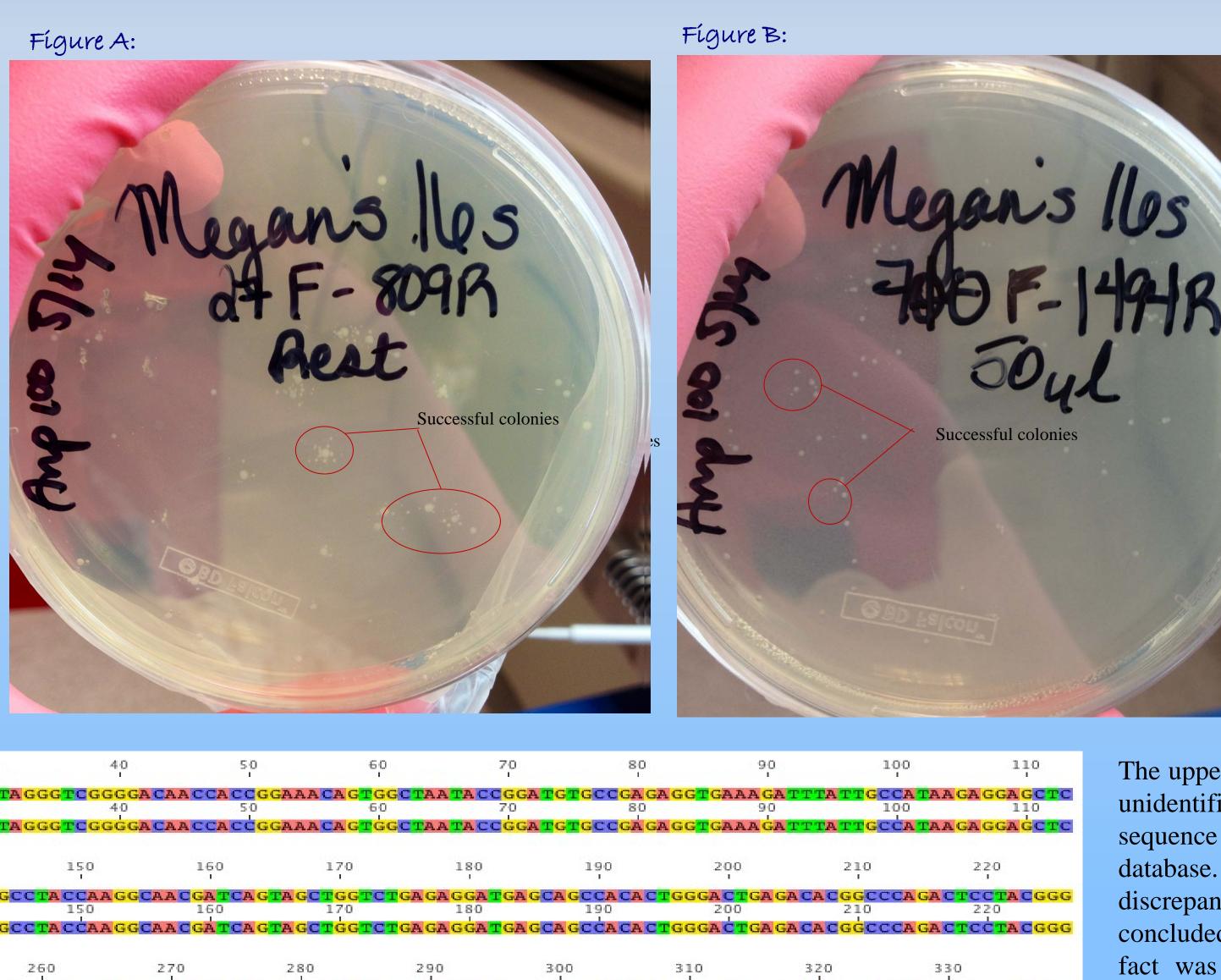
Gerwick Laboratory, Sverdrup Building, Scripps Institute of Oceanography and Marine Biology.

Protocol Outline

DNA Isolation and PCR

Transformation of E.coli

In order to mass-replicate the vector containing the gene of interest, the vectors were placed in chemically competent E.coli cells, which are capable of such mass replication without altering the DNA sequence. Since two sets of primers were used (27 forward, 809 reverse; and 740 After DNA dilutions of 1:1, forward, 1494 reverse), each of the samples were separately 1:10, 1:50, and 1:100 were replicated in order to complete the entire gene. This split in made, PCR proved to be the gene of interest is fixed when the sequences are successful for 1:10 and 1:50. obtained. Figure A represents the successful transformation This means that the correct of the E.coli containing the 27 to 809 base pair vector. of DNA was Figure B represents the successful transformation of the 740 amplified, followed by cloning to 1494 base pair vector. Figure C represents negative of the PCR products using the control using bacteria not containing the vector, which also TOPO cloning (Invitrogen), allows for ampicillin resistance, was used to verify the hence, allowing the project to functionality of the ampicillin antibiotic on the plates. Five continue to transformation of of the colonies on each successful plates were cultured in the resulting plasmid into TOP individual tubes, and then treated with PCR to further replicate the vector. Lastly, the vector was isolated and cleaned to be sent to the Retrogen© lab for sequencing. Sequences were received the next morning and analyzed as an halou



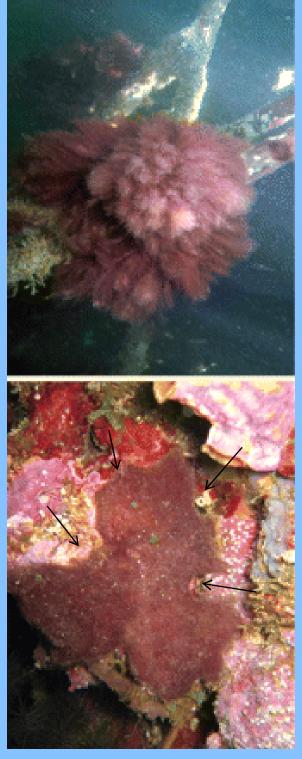
seen below.	
Comparing and	Identifying

	1	10	20	30	40	50	60	70	80 '	90	100	110
EMILY MEGAN CONSENSUS		GTAACGCO	GTGAGAAT(CTGCCTTAGG	GTCGGGGA	CAACCACCGGA	AACAGTGGCTA	A TA CCGGA TG	TGCCGAGAGGT 80	GAAAGATTTAT 90	TGCCA TAAGA	GGAGCTC 110
M.BOUILLONII 165	GACGGG <mark>T</mark> GA	GTAACGCC	STGA GAA T	CTGCCTTA GO	GTCGGGGGA	CAACCACCGGA	AACAGTGGCTA	A TA CCGGA TG	TGCCGAGAGGT	GAAA GA TTTA T	TGCCA TAA GA	GGAGCTC
EMILY MEGAN CONSENSUS	120		30	140	150		170			200 2	1	20
M.BOUILLONII 165	GCGTCTGAT 120	TAGCTAG 13						180				
M.BOOILLONII 105	000101041	IRGUIRG.	11001000						<u></u>		Geeendreie	e Incouo
EMILY MEGAN CONSENSUS	230 AGGCAGCAG				AGCCTGAC			CAAGGCTTTT	310	320 1	330	AAGTGAC
M.BOUILLONII 165	230	240			AGCCTGAC	270		GAAGGCTTTT	GGGTTGTAAAC	CTCTTTTATCA	330	AAGTGAC
				370	200	200	100		130	120		
EMILY MEGAN CONSENSUS	340 GGTACCTGA	350	360	370		390	400	410	420			450 ACCTCAA
M.BOUILLONII 165	340	350	360	370	380	390	400 GGAGGATGCAA	410 GCGTTATCCG	420 GAATTATTGGG	430 CGTAAAGCGTC	440	450
	450		470	490	400	500	510	520	F 3.0	540	550	560
EMILY MEGAN CONSENSUS	460 GTCGGCTGT		470 	480 AACTCTGGGG	490 AGGCAGTGO	500 GAAACTGAGG	510 AACTAGAGGGCA	520 GTAGGGGTAG	530 AGGGAATTCCC	540 GGTGTAGCGGT	GAAATGCGTA	560 GATTTCG
M.BOUILLONII 165	460 GTCGGCTGT		470	480	490	500 GAAACTGAGGA	510	520	530 AGGGAATTCCC	540 GGTGTAGCGGT		560
	570	580	59	30 6	500	610	620 6	30 6	40 65	0 660	670	
EMILY MEGAN CONSENSUS	GGAA GAA CA	TCGGTGG	GAAGGCG	CTCTACTGGA	CTGAACCT	GA CA CT GA GG	GACGAAAGCTAG	GGGAGCGAAT	GGGA TTA GA TA	CCCCAGTAGTC	CTAGCTGTAA	ACGATGG
M.BOUILLONII 165	570 GGAA GAA CA	580	59 GAAGG <mark>C</mark> G	1	500	610 GA CA CT GA GGO	620 6 GACGAAAGCTAG	1	40 65 GGGATTAGATA		the second s	ACGATGG
	680	690	700	710	720	730	740	750	760	770	780	790
EMILY MEGAN CONSENSUS	ATACTAGGT	GTAGCTT	GTA TCGA CO	CCGAGCCGTG	GCCGAAGCM	AACGCGTTAA	TATCCCGCCTG	GGGAGTACCC	ACGCAAGTGTG	AAACTCAAAGG	AATTGACGGG	GGCCCGC
M.BOUILLONII 165	680 A TA CTA GGT	690	700 GTATCGACC	710	720 CCGAAGCC	730 AACGCGTTAAC	740	750 GGGAG <mark>TAC</mark> GC	760 ACGCAAGTGTG	770 AAACTCAAAGG	780	790 GGCCCGC
	80	0	810	820	830	840	850	860	870	880	890	900
EMILY MEGAN CONSENSUS	ACAAGCGGT	GGAGTAT	G <mark>TGGTTTA</mark>	A TTCGA TGCA	ACGCGAAGA	AACCTTACCA	GGGCTTGACATG	TCGCGAA TCC	GGTGAAAGCT	GGGAGTGCCTT		AACACAG
M.BOUILLONII 165	80 A CAA G <mark>C GG T</mark>		810 GTGGTTTAA	820 ATTCGATGCA	830 ACGCGAAGA	840	850 GGGCTTGACATG	860 TCGCGAATCC	870 CGGTGAAAGCT	880 GGGAGTGCCTT	890	900 AACACAG
	910	920	h e	930	940	950	960	970	980 9	90 1,00	00 1,01	0
EMILY MEGAN CONSENSUS	GTGGTGCAT	GGCTGTC	GTCAGCTC	GTGTCGTGAG	A TGTTGGG	TAAGTCCCG		CCCTCGTTTT	TAGTTGCCAGC	ACGTGAAGGTG	GGCACTCTAG	A GA GA CT
M.BOUILLONII 165	910 GTGGTGCAT	920 GGCTGTC	GTCAGCTC	930 GTGTCGTGAG	940 ATGTTGGG	950	960 CAACGAGCGCAA	970 CCCTCGTTTT	980 9 TAGTTGCCAGC	90 1,04 ACGTGGTGGTG		A GA GA CT
	1,020	1,030	1,040	1,050	0 1,0	60 1,0	70 1,080	1,090	1,100	1,110	1,120	1,130
EMILY MEGAN CONSENSUS	GCCGGTGAC		GGAAGGT	GGGGATGAC	TCAAGTCAG	GCATGCCCCT	ACGCCCTGGGC	GA CA CA C GTA	CTA CAA TGGTC		CAGCCAACTC	
M.BOUILLONII 165	1,020 GCCGGTGAC	1,030	1,040 AGGAAGGT	1,050 GGGGATGAC) 1,0 GTCAAGTCAC		70 1,080	1,090 TACACAC <mark>GT</mark> A	1,100 CTA CAA T <mark>GGTC</mark>	1,110 AGGACAATGGG	1,120 CAGCCAACTC	1,130 GCAAGGG
	1,	140	1,150	1,160	1,170	1,180	1,190	1,200	1,210	1,220	1,230	1,240
EMILY MEGAN CONSENSUS	GGAGCTAAT	CTCA TCAP	ACCTGGCC	TCAGTTCAG	ATTGCCGG	TGCAACTCG	CCGGCATGAAGG	AGGAA TCGCT.	AGTAATCGCCG	GTCA GAA TA C G	GCGGTGAATC	C <mark>GTT</mark> CCC
M.BOUILLONII 165		140	1,150 AACCTGGCC	1,160	1,170	1,180 CTGCAACTCG	1,190 CCGGCATGAAGG	1,200 AGGAATCGCT	1,210 AG <mark>TAA TCGCCG</mark>	1,220 GTCAGAATACG	1,230 GCGGTGAATC	1,240 CGTTCCC
	1,250	1,2	60	1,270	1,280	1,290	1,304					
EMILY MEGAN CONSENSUS	GGGCCTTGT	ACACACC	GCCCGTCA	CACCATGGAA	GCTGGCCA	GCCCGAAGT	GTTACCCT					
M.BOUILLONII 165	1,250 GGGCCTTGT	and the second second second second	GCCCGTCA	1,270 CACCATGGAA	1,280	1,290 1,294						



Cloning, Transformation, and Plasmid Extraction

The upper strand shown is the 16s rRNA sequence for the Papua New Guinea unidentified cyanobacteria. The lower strand is the general 16s rRNA sequence for the Moorea Bouillonii species, as provided by the NCBI database. If the two strands were followed base-pair by base-pair, no discrepancy between the two sequences would be found. From this it can be concluded that the unidentified cyanobacteria is, in fact, a M.bouillonii. This fact was already suspected as a result of the richness of its secondary metabolite production; a morphological identification at the time of collection. M.bouillonii is a tropical cyanobacterium that is filamentous and often form large, mat-like colonies¹. This finding is valuable to continued research on this useful organism.



Underwater image of a large tuft of Moorea Bouilonii

Analyzing the Sequences

Figure C:



Acknowledgements

I'd like to thank Dr.Komives for creating this program.

To William and Lena Gerwick for allowing me to work in their lab and for helping me continuously throughout my stay.

To Emad Alazzeh for his help in the lab.

To Emily Mevers for her helpful information on her research with apratoxin A and the amino glycerol compound, as well as her willingness to help explain her research.

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1. Engene, N. et. al. and Gerwick, WH (2011) Phylogeny-Guided Isolation of Ethyl Tumonoate A from the Marine Cyanobacterium cf. Oscillatoria margaritifera. Journal of Natural Products 74, 1737-1743.