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Two Diverse Constituents of the Cephalaspidean Mollusk *Smaragdinella calyculata*[1]

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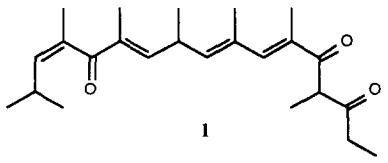
ABSTRACT: Isolation of an aminoalkylpyridine, naloamine (**4**), and a cytotoxic polypropionate, nalodionol (**5**), from *Smaragdinella calyculata* is described. The structures were determined by interpretation of spectral data. Copyright © 1996 Elsevier Science Ltd

Cephalaspidean mollusks (Order Cephalaspidea, Subclass Opisthobranchia, Class Gastropoda) embrace carnivorous and herbivorous families.[3] Some years ago we studied the constituents of the carnivore *Philinopsis speciosa* (Family Aglajidae), from which we isolated two polypropionate metabolites, niuhinone A (**1**) and B (**2**) and a much less common polyketide substituted α-pyridine, pulo'upone (**3**).[4,5] At the same site, Pupukea on



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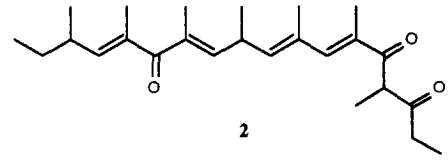

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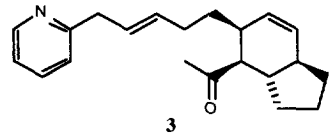

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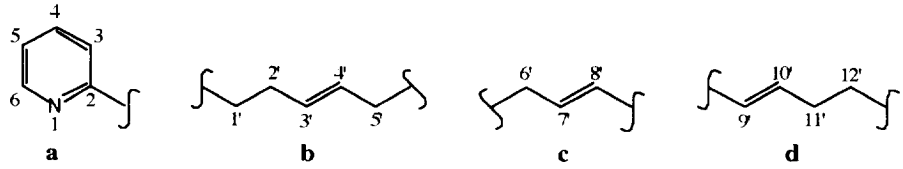
the north shore of O'ahu, we recently collected specimens of *Smaragdinella calyculata*, which is an herbivorous cephalaspidean in the Family Smaragdinellidae. Again, despite the radically different diets of these two taxa, we isolated from this mollusk a polypropionate and an α-substituted pyridine. In a study of Mediterranean opisthobranch mollusks Avila[6] suggests that pyridine (occasionally benzene-substituted) polyacetates play a role as alarm pheromones, while the polypropionates may act as defensive agents. In virtually all of the examples studied so far, either a polypropionate or an alkylpyridine, but not both types, were found.[6]

S. calyculata were collected during the summer months of 1994 and 1995 in the tidepools of Pupukea beginning just before sunset, when the green cryptic animals appear on lava rocks. The earlier collection was freeze-dried, extracted with ethyl acetate and purified by size exclusion, flash, and HP liquid chromatography, yielding a colorless solid of composition $C_{17}H_{24}N_2$, supported by HR-EIMS data of the peak at *m/z* 226.1576

$[C_{16}H_{20}N$ (Δ -2.0 mmu)] $(M\text{-}CH_2NH_2)^+$. Early structural clues, in addition to obvious chemotaxonomic implications, included a substantial peak in the EI mass spectrum at *m/z* 226, indicating a loss of 30 amu from the insignificant molecular ion at *m/z* 256, representing α-cleavage of a primary amine.[7] A prominent mass peak at *m/z* 79 corresponding to $C_5H_5N^+$ and a small peak at *m/z* 78 suggested a molecule with a substituted pyridine and a methyleneamine terminus (Fig. 1). These features were confirmed by a broad IR band at 3400 cm^{-1} and UV maxima at 262, 268, and 280 nm, respectively. Interpretation of ^1H and ^{13}C NMR spectra readily elucidated the hydrocarbon fragment of the molecule, $C_{12}H_{18}$, with three double bond equivalents. This was confirmed by six olefinic methines, which gave rise to signals between 5 and 6 ppm and between 130 and 133 ppm (see Table 1). Interpretation of the COSY spectrum allowed complete structure assignments via four partial structures, **a** - **d**. Partial structure **b** arises from joining the two unassigned sp^2 carbons, C3' and C4' based on the proton coupling constants. The downfield shifts of H-8' (δ 5.92) and H-9' (δ 5.99) were diagnostic for a conjugated diene, which connected partial structures **c** and **d** from C-8' and C-9'. A UV band at 230 nm provided additional evidence. Two of the six methylene resonances are complex multiplets at 2.01 and 2.03 ppm (H_2-5' and H_2-6') connecting partial structure **b** with **c** - **d**. Of the four remaining methylene groups, H_2-12' was confirmed by preparing the acetamide of the free amine, which, without purification, shifted the H_2-12' triplet from 2.55 to 2.82 ppm, thus completing the structure as 2-(12'-amino-3', 7', 9'-dodecatrienyl)-pyridine (**4**), for which we have chosen the trivial name naloamine.[8] The geometry of the three double bonds is *E* on the basis of the values for the coupling constants: $J_{3'\text{-}4'}$ = 15.1 Hz, $J_{7'\text{-}8'}$ = 14.6 Hz, and $J_{9'\text{-}10'}$ =14.5 Hz.



a **b** **c** **d**

Table 1. ^1H and ^{13}C Data of Naloamine (**4**) (CD_3OD)

Number	^{13}C (ppm)	^1H (ppm)	multiplicity	*J* (Hz)	Integration	COSY
1	-----	-----				
2	162.6	-----				
3	124.8	7.28	dl	7.9, 1.0	1H	H4
4	138.6	7.73	ddl	7.9, 7.4, 1.9	1H	H3, H5
5	122.8	7.23	ddl	7.4, 5.1, 1.1	1H	H4, H6
6	149.4	8.41	ddl	5.1, 1.9, 1.0	1H	H5
1'	38.7	2.82	dl	7.5, 7.7	2H	H2'
2'	33.9	2.38	bddt	7.7, 6.2, 7.5	2H	H1', H3'
3'	130.4	5.45	dt	15.1, 6.2	1H	H2'
4'	131.8	5.39	dt	15.1, 5.5	1H	H5'
5'	33.4	2.01	m		2H	H4'
6'	33.6	2.03	m		2H	H7'
7'	133.1	5.50	dt	14.6, 7.4	1H	H6', H8'
8'	131.9	5.92	ddt	14.6, 10.2, 1.2	1H	H7'
9'	132.5	5.99	ddt	14.5, 10.2, 1.2	1H	H10'
10'	131.2	5.51	dt	14.5, 7.3	1H	H9', H11'
11'	27.7	2.28	bdt	7.4, 7.3	2H	H10', H12'
12'	43.9	2.55	t	7.4	2H	H11'

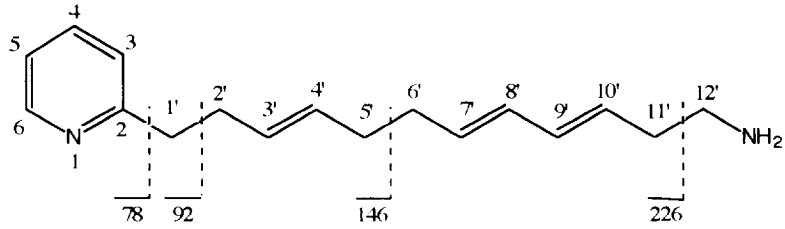
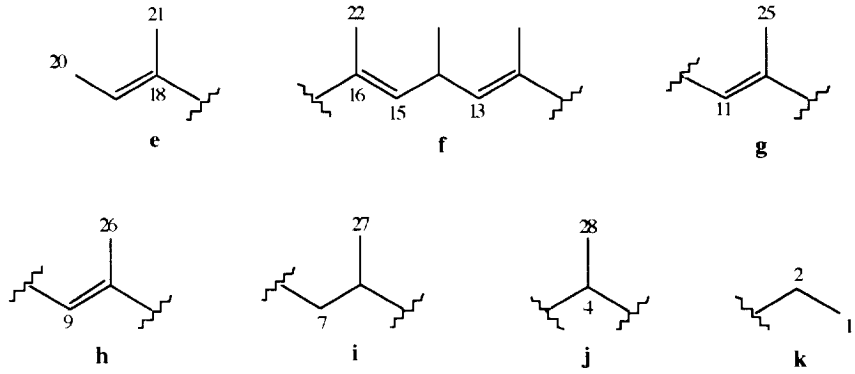


Fig 1. Mass fragmentation pattern of naloamine (**4**).

The same mollusks were recollected in 1995. The MeOH extract of the specimens was partitioned between hexane and H_2O, and the organic layer was subjected to High Speed Counter Current Chromatography (HSCCC) (heptane/CH_2Cl_2/MeCN, 10:3:7) and ODS HPLC [COSMOSIL $5C_{18}$-AR, (85% MeCN)] to yield a single peak of polypropionate compounds. Interpretation of the COSY spectrum of **5** disclosed the presence of partial structures **e** - **k**, of which **i**, **j**, and **k** were separated by carbonyl groups. The ^1H and ^{13}C NMR spectra showed doubled signals, as if there were two compounds. Therefore, this fraction was further purified on a linked ODS column [COSMOSIL-$5C_{18}$ MS and AR, (75% MeCN)]. In the final separation, two partially overlapping peaks (fractions 1 and 2) were observed, and collected carefully so as to avoid the overlapping portions.

However, the ^1H-NMR spectra of fractions 1 and 2 were superimposable; each consisted of paired signals in 1:1 ratio. Therefore, we concluded that **5** existed in two interconverting forms and interpreted the spectra of the binary mixture, which exhibited doubled ^1H and ^{13}C signals of fragments **g** - **k**.



These partial structures were connected on the basis of HMBC correlations, which are shown by curved arrows in the Fig. 2. Although there were no apparent signals due to carbonyl carbons in the ^{13}C NMR spectrum, Me-1 and Me-27 showed clear 3-bond H-C correlations to the C-3 and 5 carbonyl carbons, respectively. Both carbonyl carbons correlated with Me-28 methyl protons, thus connecting fragments **i**, **j**, and **k**. Fragments **e** and **f** were connected through the H-17 oxymethine proton (4.22 ppm), which showed perfect correlations to C-15, 16, 18, 19, 21, and 22. Partial structure **h** was connected to C-7 of **i** by the correlations between H-9/C-7 and H-26/C-7. Fragment **g** was placed between **f** and **h** on the basis of three bond C-H

correlations among H-9/C-25, H-11/C-13, and H-13/C-11, completing the structure of **5**. HR-EIMS showed only a dehydro molecular ion peak at *m/z* 410.3202 [$C_{28}H_{42}O_2$ (Δ +1.8 mmu)], but the positive ion FAB-MS showed a $(M+H)^+$ ion peak at *m/z* 429 corresponding to a molecular formula of $C_{28}H_{44}O_3$, which is in agreement with structure **5**. The 1,3-diketone functionality of **5**, which causes keto-enol tautomerization and leads to racemization of C4, offers a reasonable explanation for the doubled signals. Thus, compound **5** is 17-hydroxy-4,6,8,10,12,14,16,18-octamethyl-8,10,12,15,18-eicosapentane-3,5-dione, which we have called nalodionol. Interestingly, naloamine was not found in the extract of the animals obtained from the second collection.

Table 2. ^1H and ^{13}C Data of Nalodionol (**5**) (CD_3CN)

C#	^{13}C (ppm)	^1H (ppm)	multiplicity	*J* (Hz)	^{13}C (ppm)	^1H (ppm)	multiplicity	*J* (Hz)
	Epimer 1				Epimer 2			
1	7.9	0.94	t	7.2	8.0	0.96	t	7.2
2	35.8	2.46, 2.44	m		35.7	2.50	q	7.2
3	209.0				209.3			
4	59.5	3.94	q	7.1	59.5	3.90	q	7.1
5	212.4				212.3			
6	44.8	2.95	qdd	6.4, 6.6, 6.4	45.1	2.92, 1.92	qdd	6.4, 6.6, 6.4
7	44.6	2.40, 1.94	ddd,m	0.7, 6.6, 13.1	45.0	2.33, 1.92	ddd,m	0.7, 6.4, 13.0
8	134.2				134.2			
9	133.1	5.68	bs		133.1	5.64	bs	
10	133.1				133.1			
11	134.1	5.67*	bs		134.0	5.66*	bs	
12	131.5				131.5			
13	136.1	5.20	dm	9.0	136.1	5.20	dm	9.0
14	32.7	3.38	ddq	9.0, 9.0, 6.7	32.7	3.38	ddq	9.0,9.0, 6.7
15	130.7	5.35	ddq	9.0, 1.3, 1.3	130.7	5.35	ddq	9.0, 1.3, 1.3
16	134.8				134.8			
17	81.6	4.22	bs		81.6	4.22	bs	
18	137.4				137.4			
19	120.7	5.51	bq	6.7	120.7	5.51	bq	6.7
20	13.3	1.59	bd	6.7	13.3	1.59	bd	6.7
21	11.9	1.41	bs		11.9	1.41	bs	
22	12.8	1.47	d	1.3	12.8	1.47	d	1.3
23	21.9	1.02	d	6.7	21.9	1.02	d	6.7
24	17.4	1.74	bs		17.4	1.74	bs	
25	19.1	1.82	d	1.3	19.1	1.81	d	1.5
26	18.0	1.76	d	1.3	18.0	1.77	d	1.3
27	16.7	0.98	d	6.4	16.5	1.00	d	6.4
28	13.2	1.20	d	7.1	13.0	1.16	d	7.1

* Assignments may be interchanged.

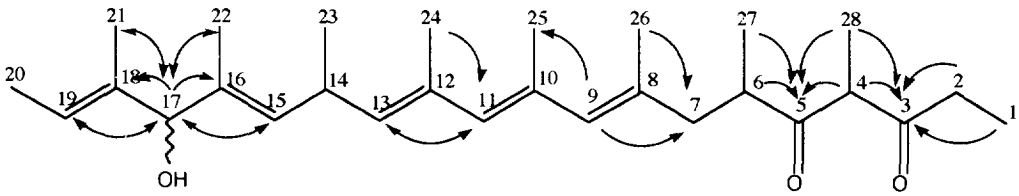


Fig. 2. Nalodionol (**5**). HMBC correlations shown by arrows.

Nalodionol (**5**) showed cytotoxicity against P388 mouse leukemia cells at an IC_{50} of 3.5 μg/mL.

EXPERIMENTAL SECTION

General Experimental Procedures

UV absorption spectra were recorded on a Hewlett Packard 8452A spectrophotometer. ^{1}H-NMR spectra were recorded at 500 MHz and ^{13}C NMR spectra at 125 MHz on a General Electric GN Omega 500 NMR spectrometer. EI-MS data were obtained with a VG70/SE mass spectrometer. FAB-MS were recorded on a JEOL SX102/SX102 tandem mass spectrometer using glycerol as a matrix.

Collection and Isolation

Animals were collected at Pupukea, O'ahu, Hawai'i, during the summer of 1994. The animals appeared on the rocks at sea level just prior to sunset. The animals were identified as *Smaragdinella calyculata* (Broderip and Sowerby, 1829) by Professor E. A. Kay, University of Hawai'i. The freeze-dried material (130 g, 2084 animals) was extracted with 4x500 mL ethyl acetate.

After removal of the solvent *in vacuo*, the extract was fractionated by gel permeation chromatography on an LH-20 Sephadex column using MeOH/CH_2Cl_2 (1:1) followed by ODS flash chromatography (60 to 100% MeOH, 10% stepwise gradient) and reverse phase HPLC using MeOH/H_2O (4:1, 2 mL/min) as the mobile phase. This was repeated on the same column with MeOH/H_2O (3:1, 1 mL/min) to give 1.0 mg of compound **4** as a colorless solid.

In search for other constituents of *S. calyculata*, the mollusks were recollected (1500 animals, corresponding to a dry weight of 94 g) in 1995. The animals were extracted with methanol (4 x 500 mL). The extract was partitioned between water and hexane. The organic layer was subjected to HSCCC, heptane/CH_2Cl_2/CH_3CN (10:3:7). The polypropionate-containing fraction was purified by ODS HPLC with CH_3CN/H_2O (85:15). This was repeated, on the same column with CH_3CN/H_2O (3:1) to afford 1 mg of compound **5** as a colorless solid. In order to isolate more of this compound, the freeze-dried animals (50.17 g, 800 animals) were extracted with 700 mL of hexane. The extract was subjected to flash chromatography (100% hexane to 80% hexane in ethyl acetate, 10% stepwise elution) on a silica gel column. Further fractionation was accomplished by ODS flash chromatography (70% to 90% CH_3CN, 10% stepwise elution). The polypropionate compound **5** was purified by ODS HPLC with CH_3CN/H_2O (4:1) and repeated with CH_3CN/H_2O (3:1) as the eluents. This yielded an additional 2.4 mg of **5**.

Naloamine (4). Colorless amorphous solid. UV (MeOH) λ_{max} 206 (ε 12000), 230 (15000), 262 (4300), 268 (4200), and 280 (2700); EIMS *m/z* (relative intensity) 78 (3), 79 (19), 92 (6), 93 (22), 117 (16), 118 (11), 146 (100), 226 (8), and 255 (0.3); HR-EIMS *m/z* 226.1576 [$C_{16}H_{20}N$ (Δ -2.0 mmu)] (M-CH_2NH_2)$^+$; IR ($CHCl_3$) 3400, 1740 cm^{-1}; ^{1}H and ^{13}C-NMR data in Table 1.

Naloacetamide. Naloamine (**4**) (0.5 mg) was acetylated with 50 μL of acetic anhydride in 50 μL of pyridine. Acetic anhydride and pyridine were dried over $MgSO_4$ prior to use. The reaction mixture was stirred at room temperature for three days. ^{1}H-NMR of the crude product was recorded; the diagnostic H_2-12' triplet was shifted from δ 2.55 to 2.82 ppm.

Nalodionol (5). Colorless amorphous solid. $[\alpha]_D$ +91° (MeOH, *c* = 1.2) UV (MeOH) λ_{max} 207 (ε 1100) and 267 (9500). FAB-MS *m/z* 411 (M-H_2O+H)$^+$, 429 (M+H)$^+$, 451 (M+Na)$^+$, 467 (M+K)$^+$; IR ($CHCl_3$) 1727, 1698 cm^{-1}; and ^{1}H and ^{13}C-NMR data in Table 2.

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REFERENCES AND NOTES

1. A preliminary account was presented at the Gordon Research Conference on Marine Natural Products in Ventura, CA, in February, 1996.
2. Howard Hughes Medical Institute Undergraduate Research Fellow.
3. Kay, E. A. *Hawaiian Marine Shells*. Bernice P. Bishop Museum Special Publication 64 (4). Bishop Museum Press: Honolulu, HI, 1979; pp 417-432.
4. Coval, S. J.; Schulte, G. R.; Matsumoto, G. K.; Roll, D. M.; and Scheuer, P. J. *Tetrahedron Lett.* **1985**, *26*, 5359.
5. Coval, S. J.; Scheuer, P.J. *J. Org. Chem.*. **1985**, *50*, 3024 - 3025.
6. Avila, C. *Sci. Mar.*. **1992**, *56*, 373-382.
7. Pretsch, E.; Clerc, T.; Seibl, J.; Simon, W. *Tables of Spectral Data for Structure Determination of Organic Compounds*, Second Ed., Springer-Verlag: Berlin, 1989; p M10.
8. The name is derived from the Hawaiian word *nalo*, which means hidden and refers to the cryptic nature of the animals.

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