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New Acetylenic Metabolites from the Marine Sponge *Pellina triangulata*[1]

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ABSTRACT

Six new acetylenic compounds, **1**, **7**-**11**, were isolated from the marine sponge *Pellina triangulata*. Structures were established using NMR spectroscopy and chemical degradation. Collisional activation decomposition (CAD) tandem mass spectrometry of the lithium adducts of the acetylenic compounds was applied to ascertain if charge-remote fragmentation would yield definitive information regarding the site of internal unsaturation in these polyfunctional compounds. Pellynic acid (**1**) inhibited inosine monophosphate dehydrogenase (IMPDH) *in vitro*.

INTRODUCTION

In earlier papers we reported the isolation from sponges of several phenols[2] and brominated tyrosine tetramers (bastadins)[3] which displayed an *in vitro* inhibitory effect on inosine monophosphate dehydrogenase (IMPDH) in a screening program designed to detect potential leads for anticancer drug discovery. Our attention was drawn to extracts of the sponge *Pellina triangulata* by similar inhibitory action on IMPDH and we report here the isolation and structure elucidation of a new acetylenic acid (**1**) which inhibits IMPDH *in vitro*, plus several related, new alcohols which are inactive in the assay. Acetylenic compounds with a variety of biological activities have previously been isolated from marine sponges of the genera *Petrosia* and *Xestospongia* which belong to the order Haplosclerida.[4-16] Although the genus *Pellina* also belongs to the order Haplosclerida, no acetylenic compounds have been reported from this genus to date.[17] Earlier chemical and biological investigation of sponges of the genus *Pellina* (order Haplosclerida, family Oceanapiidae) led to the isolation of antimicrobial alkaloids[18], carcinogenic aromatic amines[19], and steroids.[20]

RESULTS AND DISCUSSION

Samples of *Pellina triangulata* were obtained from Chuuk Atoll and extracted with MeOH and CH_2Cl_2 to give extracts that inhibited IMPDH activity. The combined, concentrated extracts were subjected to Kupchan partitioning[21] to give hexane, $CHCl_3$, *n*-BuOH and H_2O-soluble fractions. The $CHCl_3$ solubles were chromatographed on silica gel open column and reverse phase HPLC to afford an IMPDH-active acetylenic acid, pellynic acid (**1**), the acetylenic alcohols, pellynols A-D (**7**-**10**) and pellynone (**11**).

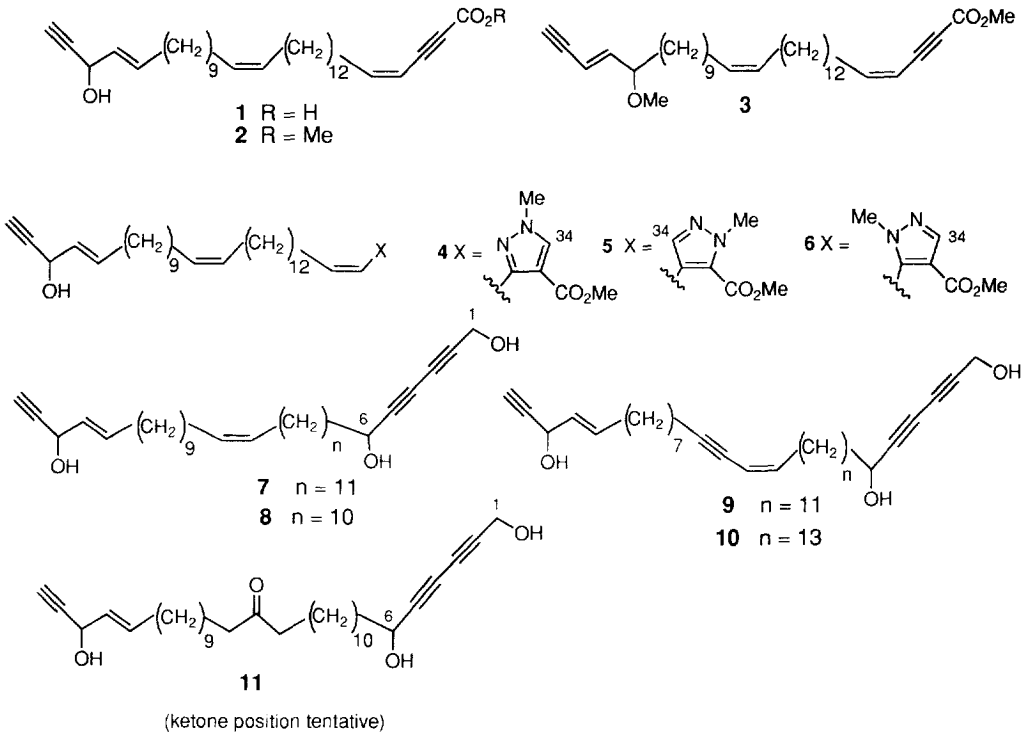
The ^1H NMR spectra of all the isolates contained features reminiscent of the spectra of the acetylenic compounds described from sponges[4-16] and this facilitated the structure elucidations. Pellynic acid (**1**), was

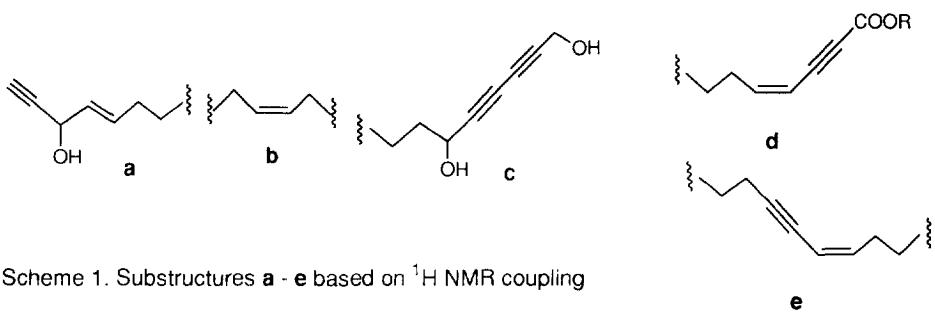
obtained as gum, $[\alpha]_D$-10.5° (c 0.34, $CHCl_3$:MeOH 1:1). The positive ion high resolution fast atom bombardment mass spectrum (HRFABMS) [calc'd for $(M + K)^+$ 535.3554; obs, 535.3553] and negative ion FABMS [*m/z* 495 $(M - H)^-$] of pellynic acid considered together with ^{13}C NMR and IR data confirm a molecular formula of $C_{33}H_{52}O_3$. The IR spectrum shows absorptions at 3560, 3290, and 2210 cm^{-1} for hydroxyl, acetylenic CH, and triple bond, respectively, and an intense band at 1585 cm^{-1} indicative of a *carboxylate* group rather than a free acid moiety. A ^{13}C NMR absorption at δ 161.4(s) was consistent with this inference. The free acid was obtained by washing a chloroform solution of the organic salt with dilute HCl and recovering the organic material, IR 3300, 2210, and 1686 cm^{-1}. IR absorption at 1585 cm^{-1} corresponding to the carboxylate group was also reported for nepheliosyne.[12] Acetylenic acid **1** was cleanly converted to the methyl ester **2** upon treatment with CH_2N_2 in ether for a short time (5 minutes). By comparing the ^1H and ^{13}C NMR spectra of pellynic acid (**1**), see Table 1, with those of the known acetylenic alcohol, melyne A[14], partial structures **a** and **b** (Z geometry assigned from ^{13}C δ 27.6 for allylic carbons) were easily recognized, see Scheme 1. The NMR signals for substructure **c** found in melyne A were missing in the spectrum of pellynic acid (**1**) and instead signals for substructure **d** (R=Na) were found. The NMR data for substructure **d** (R=Me) in the ester **2**, see Table 1, were nearly identical to that of the same substructure in petroformyne 1[15] and corticatic acid[13] methyl esters. The above data suggest for pellynic acid a linear structure characterized by terminal units **a** and **d**, and containing a *cis* double bond (substructure **b**) between methylene chain segments of undetermined lengths. ^1H-^1H COSY, RELAY-2 and HETCOR experiments confirmed these conclusions. The lengths and relative positions of the $-(CH_2)_n$ units were determined by comparing oxidative degradation products of pellynic acid with those of the rearranged ester **3**. This ester, wherein the allylic alcohol of **1** has undergone allylic rearrangement and concomitant ether formation, see Table 1, was obtained in good yields either by refluxing **1** in methanol with BF_3 as catalyst[22] or treating **1** at room temperature with methanol/chlorotrimethylsilane.[23] The methoxy group in **3** "labels" the adjacent methylene chain in oxidative-degradation experiments.

Ozonolysis of **1** followed by H_2O_2 oxidation and methylation of the resulting mixture of acids with CH_2N_2 afforded a mixture which GC/MS analysis revealed contained two major components whose mass spectra correspond to expectations for dimethyl undecandioate,[24] *m/z* 213 $[M - OCH_3]^+$, and dimethyl tetradecandioate, *m/z* 255 $[M - OCH_3]^+$. Degradation of **3** in the same manner followed by GC/MS analysis resulted in the observation of two major GC peaks corresponding to dimethyl tetradecandioate, *m/z* 255 $[M - OCH_3]^+$, and dimethyl 2-methoxydecandioate, *m/z* 229 $[M - CO_2CH_3]^+$, 197 $[M - MeOH - CO_2CH_3]^+$ and 104.[25] Thus the $(CH_2)_9$ unit is at the distal end of acid **1**.

Although treatment of **1** with CH_2N_2 for a brief period gave the ester **2**, exposure to excess CH_2N_2 for longer times (i.e. overnight at room temperature) resulted in 1,3-dipolar addition to the conjugated acetylenic group followed by prototropic shifts and methylation[26] to give **4**, **5**, and **6** in a ratio of 3:4:2, respectively, as estimated by the integration of the ^1H NMR spectrum of the reaction mixture. The ^1H NMR spectra of **4**, **5**, and **6** are virtually identical to that of **2**, see Table 1, for the H-6 to H-33 structural element. However, the signals for H-4 and -5 in **2** were missing in the spectra of **4** and were replaced by signals at δ 6.77 (dt, J = 11.6, 1.2 Hz, H-4) and 5.84 ppm (dt, J = 11.6, 7.3 Hz, H-5). The olefinic signal of the ring occurred at δ 7.87 (s) ppm and the N-methyl at 3.90 ppm. Similar absorptions are present in the spectra of **5** and **6**, see Experimental Section.

Although four regioisomers would be expected[26b] for the acetylenic ester in the 1,3-dipolar cycloaddition reaction and methylation with diazomethane, only three, **4-6**, were isolated by reverse phase HPLC. The





Scheme 1. Substructures **a** - **e** based on ^{1}H NMR coupling

Table 1. ^{1}H and ^{13}C Chemical Shifts for **1**-**3**[a,b]

No.	Pellynic Acid (**1**)		**2**		**3**	
	$^{1}H(J,Hz)$	^{13}C[c]	$^{1}H(J,Hz)$	^{13}C[c]	$^{1}H(J,Hz)$	^{13}C[c]
1		161.4(s)		154.4(s)		154.5(s)
2		77.6(s)		83.7(s)		83.8(s)
3		88.5(s)		84.2(s)		84.3(s)
4	5.46(bd,10.7)	106.9(d)	5.54(d,10.9)	106.3(d)	5.52(dt,10.9,1.4)	106.4(d)
5	6.03(dt,10.7,7.8)	147.3(d)	6.25(dt,10.9,7.5)	151.6(d)	6.23(dt,10.9,7.6)	151.6(d)
6	2.29(q,7.3)	30.2(t)	2.36(dq,1.0,7.4)	30.9(t)	2.35(dq,1.4,7.4)	30.9(t)
17	1.96(q,5.9)	26.7(t)	2.01(q,6.4)	27.0(t)	1.98(brdd,5.7,6.3)	27.2(t)
18	5.29(t,4.9)	129.3(d)	5.34(t,6.4)	129.8(d)	5.32(t,4.6)	129.8(d)
19	5.29(t,4.9)	129.3(d)	5.34(t,6.4)	129.8(d)	5.32(t,4.6)	129.8(d)
20	1.96(q,5.9)	26.7(t)	2.01(q,6.4)	27.0(t)	1.98(brdd,5.7,6.3)	27.2(t)
28	2.00(q,6.8)	31.5(t)	2.06(q,7.4)	31.9(t)	1.44(m),1.52(m)	29.0
29	5.82(dt,15.1,7.3)	133.1(d)	5.91(dt,15.3,6.9)	134.3(d)	3.54(q,6.7)	81.7(d)
30	5.53(ddt,15.1,6.3,1.5)	128.3(d)	5.60(dd,15.3,5.9)	128.3(d)	6.06(dd,16.1,7.4)	145.9(d)
31	4.75(bd,6.3)	61.6(d)	4.83(d,5.9)	62.6(d)	5.61(ddd,16.1,2.4,1.1)	110.4(d)
32		83.1(s)		83.3(s)		81.6(s)
33	2.64(d,1.9)	72.8(d)	2.56(d,2.0)	73.8(d)	2.87(d,2.4)	77.6(s)
$COOCH_3$			3.79(s)	52.6(q)	3.78(s)	52.6(q)
OCH_3					3.25(s)	56.6(q)

[a]In $CDCl_3$; [b]Chemical shifts for methylenic carbons: δ 28.0-30.0 ppm; for methylenic protons: δ 1.20-1.45 ppm; [c]Multiplicities determined by DEPT and HETCOR experiments

assignments of the structures for compounds **4**, **5** and **6** were made on the basis of their LR COSY and NOESY spectra. The following NOE correlations support the regiochemical assignments: for **4** NOE between N-Me and H-34; for **5** between H-34 and H-6; for **6** between N-Me and H-6. The chemical shifts of the aromatic proton singlets, δ ~7.90 for **4** and **6**, and δ 7.52 for **5** are in good agreement with those reported for similar pyrazole ester regioisomers.[26b]

The molecular formulae of pellynols A (**7**) and B (**8**) were established by HRFABMS to be $C_{33}H_{52}O_3$ and $C_{32}H_{50}O_3$, respectively, and these are identical to those of melynes A and B isolated earlier from an unidentified species of *Xestospongia*.[14] The NMR data of pellynols A (**7**), see Table 2, and B (**8**), are virtually identical to those of melynes A and B. However, ozonolysis of pellynols A and B followed by oxidation with H_2O_2 and methylation of the resulting dicarboxylic acids with CH_2N_2 afforded in each case dimethyl undecandioate as a major component as indicated by GC/MS analysis [*m/z* 213 $(M - OCH_3)^+$ for the major GC peak]. Therefore pellynol A (**7**) was assigned a Δ^{19} double bond (*vs* Δ^{18} in melyne A) and pellynol B (**8**) a Δ^{18} double bond (*vs* Δ^{17} in melyne B).

Pellynol C (**9**) was obtained as a gum, $[\alpha]_D$-11.2° (c 2.38, $CHCl_3$). HRFABMS showed a peak at *m/z* 515.3496 $[M + Na]^+$, corresponding to a molecular formula of $C_{33}H_{48}O_3$ to within 1 ppm. Analysis of NMR data, see Table 2, including ^1H-^1H COSY, RELAY-2 and HETCOR spectra, revealed the substructures of pellynol C to be **a**, **c** and **e**, see Scheme 1. The ^1H and ^{13}C NMR data assigned for these substructures are in accordance with those of substructures existing in melyne A[14] and methyl 18-bromooctadeca-(9Z, 17E)-diene-7,15-diynoate.[16] The length of the methylene chain that is flanked by double and/or triple bonds was determined by GC/MS analysis of the mixture of products obtained from oxidative degradation (O_3; H_2O_2; CH_2N_2). This GC/MS chromatogram contained a major peak whose mass spectrum corresponded to dimethyl nonandioate, *m/z* 185 $[M - OCH_3]^+$. Summation of the elements confirmed by the above data reveals that the methylene chain on the right half of the molecule is 11 carbons long.

Pellynol D (**10**), $C_{35}H_{52}O_3$ by HRFABMS, differed from pellynol C (**9**) by two CH_2 units. The ^1H and ^{13}C NMR spectra of **9** and **10** were nearly identical. GC/MS analysis of the products from oxidative degradation (O_3; H_2O_2; CH_2N_2) of pellynol D yielded a major GC/MS peak exhibiting an *m/z* 185 which corresponds to $[M - OCH_3]^+$ of dimethyl nonandioate and thus supports structure **10** for pellynol D.

Pellynone (**11**), $[\alpha]_D$-9.0° (c 0.45, $CHCl_3$), was assigned the molecular formula $C_{33}H_{52}O_4$ from HRFABMS and NMR data. The latter was consistent with the presence of five non-protonated acetylenic carbons (δ 68.8(s)-83.4(s)) and one protonated sp hybridized carbon (δ C 73.9 d, δ H 2.56), thus establishing the presence of three triple bonds. One disubstituted double bond (δ C 134.5 d, 128.4 d; δ H 5.78, 5.50), one ketone (δ C 211.7) and three oxygenated carbons (δ C 51.4 t, 62.8 d, 62.8 d; δ H 4.34, 4.43, 4.83) were also confirmed from the NMR data. The presence of substructures **a** and **c** were evident from analysis of ^1H-^1H COSY, RELAY-2, and HETCOR experiments, see Table 2. A keto group in pellynone was also confirmed by an IR band at 1712 cm^{-1}. The ^1H NMR spectrum of pellynone (**11**) was similar to that of melyne A except that the two-proton olefinic triplet at δ 5.33 in the spectrum of melyne A was absent in that of pellynone (**11**). Instead, a four-proton triplet at δ 2.38 (J = 7.3 Hz) was observed and this was assigned to two methylene groups next to the ketone. The carbons of these methylene groups resonate at δ 42.8 (C-17 and C-19) as indicated by the HETCOR spectrum. From these data pellynone was deduced to have a linear structure with the terminal units **a** and **c**, and a keto group somewhere within the methylene chain.

Ozonolysis of **11** followed by H_2O_2 oxidation and methylation of the resulting products with CH_2N_2

Table 2. ^{1}H and ^{13}C Data for Compounds **7**, **9** and **11**[a,b]

	Pellynol A (**7**)		Pellynol C (**9**)		Pellynone (**11**)	
No.	^{1}H(*J*,Hz)	^{13}C[c]	^{1}H(*J*,Hz)	^{13}C	^{1}H(*J*,Hz)	^{13}C
1	4.33(brs)	51.30(t)	4.30(brs)	51.2(t)	4.34(brs)	51.4(t)
2		77.53(s)		77.6(s)		77.6(s)
3		69.73(s)		69.7(s)		69.7(s)
4		68.78(s)		68.8(s)		68.8(s)
5		80.50(s)		80.5(s)		80.5(s)
6	4.41(t,6.6)	62.76(d)	4.39(t,6.8)	62.7(d)	4.43(t,6.5)	62.8(d)
7	1.70(m)	37.42(t)	1.67(m)	37.4(t)	1.71(m)	37.4(t)
8	1.37(m)	25.02(t)	1.38(m)	25.0(t)	1.44(m)	24.9(t)
16	d	d	d	d	1.55(m)	23.9(t)
17	2.00(dd,6.6,5.7)	27.15(t)	2.25(q,6.8)	30.0(t)	2.38(t,7.5)	42.8(t)
18	5.34(t,4.9)	129.89(d)	5.77(dt,10.7,6.8)	142.5(d)		211.7(s)
19	5.34(t,4.9)	129.86(d)	5.40(brd,10.7)	109.3(d)	2.38(t,7.5)	42.8(t)
20	2.00(dd,6.6,5.7)	27.15(t)		77.4(s)	1.55(m)	23.9(t)
21	d	d		94.5(s)	d	d
22	d	d	2.30(dt,2.0,6.8)	19.5(t)	d	d
28	2.05(q,7.1)	31.92(t)	2.04(q,6.5)	31.9(t)	2.06(q,7.0)	31.9(t)
29	5.90(dt,15.7,6.6)	134.61(d)	5.88(dt,15.5,6.5)	134.4(d)	5.91(dt,16.1,7.0)	134.5(d)
30	5.60(dd,15.7,5.7)	128.24(d)	5.56(dd,15.5,5.9)	128.3(d)	5.60(dd,16.0,6.5)	128.4(d)
31	4.83(d,6.2)	62.74(d)	4.81(brd,5.9)	62.7(d)	4.83(brd,5.5)	62.8(d)
32		83.29(s)		83.3(s)		83.4(s)
33	2.56(d,2.3)	73.97(d)	2.53(d,2.3)	73.97(d)	2.56(d,2.0)	73.9(d)

[a]In $CDCl_3$; [b]Chemical shifts for methylenic carbons: δ 28.0-30.0 ppm; for methylenic protons: δ 1.20-1.40 ppm; [c]Multiplicities determined by DEPT and HETCOR experiments; [d]Not assigned.

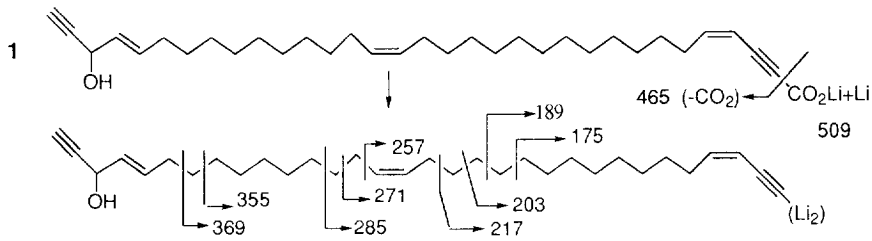
yielded a mixture of at least 10 components, one of which gave a mass spectrum upon GC/MS analysis that is nearly identical with that of dimethyl dodecandioate, as is expected for a component with the carbonyl at C-18. The dimethyl dodecandioate could be derived from cleavage of the double bond and Bayer-Villiger oxidation of the ketone followed by hydrolysis of the resulting ester and esterification with diazomethane. Complete assignment of the structure of **11** must await further experiments.

CAD TANDEM MASS SPECTRAL ANALYSIS

The central double bond location of the acetylenic compounds was also confirmed by collisional activation decomposition (CAD) tandem mass spectrometry of the lithium salt adducts. This technique has been shown to provide definitive information regarding double bond positions in simple unsaturated acids and alcohols,[27] but the technique has not yet been applied to any of the more complex long chain acetylenic acids and alcohols isolated from marine sources. Consequently, we wished to analyze the acetylenic compounds described here by CAD tandem mass spectrometry of the lithium salt adducts in order to establish if the charge-remote fragmentation would definitively determine the central double bond location. This mass spectral technique has the advantage that no derivatization is necessary and that the analysis can be carried out on small amounts of sample.

The FAB tandem mass spectrum of the $[M - H + 2Li]^+$ ion of **1** shows that the most prominent fragmentation is the loss of CO_2 to give an ion of *m/z* 465, see Scheme 2 and Table 3. At lower *m/z* values is the series of charge-remote fragments that are produced by parallel cleavages of C-C bonds with elimination of H_2 and a neutral olefin or diene. The location of the double bond can be determined from the charge-remote fragment ion series. The cleavage of homoallylic (HA) and allylic (A) bonds are facile, and as a result, relatively abundant product ions are formed. On the other hand, cleavages of vinyl (V) and double (=) bonds are not favored, and the resulting ions are of low abundance. Although the difference in mass between peaks for fragmentation along a saturated carbon chain is 14 u, the mass difference between two peaks that represent vinylic cleavages is 26 u. In the tandem or CAD mass spectrum of **1**, the fragment ions of *m/z* 285 (HA), 271 (A), 257 (V), 231 (V), 217 (A) and 203 (HA) are strong evidence that the double bond is at C-18 (see Table 3).

Scheme 2. CAD MS/MS Fragmentation Pattern for **1**.



The interpretation of the tandem mass spectrum of the triol **7** presents a small problem because the addition of the Li^+ to charge the molecule can occur at either end (see Figure 1). Because the charge site can be at one end of the molecule or the other, there are two series of charge-remote fragments; the doublets at *m/z* 253/255, 171/172, and 157/158 are consequences of these dual charge sites. Nevertheless, the position of the double bond can be determined by interpreting these two series. When the charge is on the diacetylenic side of **7**, the ion series is *m/z* 323 (HA), 309 (A), 295 (V), 269 (V), 255 (A), and 241 (HA). When the charge is on the monoacetylenic side, the ion series is *m/z* 267 (HA′), 253 (A′), 239 (V′), 213 (V′), 199 (A′), and 185 (HA′).

Table 3. Partial FAB tandem mass spectra of compounds **1**, **7-11** (in 3-NBA/LiI matrix)

Compound	FAB-MS/MS spectra - *m/z*(A%)
1[a]	465(100), 433(26), 408(2.2), 369(6.0), 355(3.4), 341(2.6), 327(1.9)[b], 313(1.7), 299(1.7), 285(5.8), 271(1.1)[c], 261(1.8), 257(1.2), 245(1.9), 231(1.9)[b], 217(3.3), 203(2.7), 189(1.5), 175(3.6), 161(4.2)[b], 147(2.2), 133(2.0), 121(1.0), 91(3.4), 77(5.9).
7	424(23), 421(17), 407(100), 393(38), 391(17), 379(24), 365(27), 351(26), 337(33), 323(49), 309(48), 295(15), 281(18), 269(6), 267(38), 255(38), 253(30), 241(18), 239(14), 227(19), 213(30), 199(39), 185(22), 172(26), 171(26), 158(31), 157(26), 143(19), 129(19), 115(17), 91(17).
8	464(43), 454(30), 433(18), 409(16) 393(100), 379(32), 365(23), 351(27), 337(22), 323(32), 309(48), 295(47), 281(28), 269(12), 267(48), 255(11), 253(45), 241(54), 239(10), 227(26), 225(13), 213(34), 199(68), 185(31), 172(44), 171(49), 158(45), 157(37), 143(30), 129(29), 115(26).
9	473(56), 463(35), 443(27), 419(22), 403(100), 389(44), 375(20), 361(19), 347(60), 333(32), 319(19), 305(12), 291(10), 277(21), 269(6), 263(67), 255(23), 249(12), 241(24), 235(7), 227(11), 213(20), 199(15), 185(10), 171(31), 158(23), 157(11), 141(20), 129(16).
10	501(51), 492(49), 431(100), 417(51), 403(21), 389(30), 375(97), 361(48), 347(45), 333(32), 319(20), 317(19), 305(20), 297(3), 291(19), 283(42), 277(42), 269(7), 263(94), 249(24), 235(19), 227(25), 215(19), 213(25), 193(18), 185(13), 181(30), 172(47), 171(29), 167(34), 158(34), 157(28), 129(32), 86(17).
11	493(32), 483(32), 463(21), 439(17), 423(100), 409(48), 395(27), 381(32), 367(41), 353(41), 339(77), 325(74), 311(14), 297(15), 283(74), 269(91), 255(26), 241(15), 227(21), 213(35), 199(32), 185(10), 175(11), 171(9), 158(11), 147(10), 119(10), 105(5), 91(5).

[a]Peaks of <1.8% were not recorded in the table unless they were identified as part of a charge-remote fragment series.

[b]Ions were absent in the spectrum taken on the array detector because they fell in the gap between the two elements of the array detector, but they were observed at smaller abundance by using a point detector. The abundances were normalized in the same way for each spectrum. The remainder of the spectrum was that taken with the array.

[c]The abundance of the ion *m/z* 271 for compound **1** is discriminated because the peak fell into the gap of the array detector.

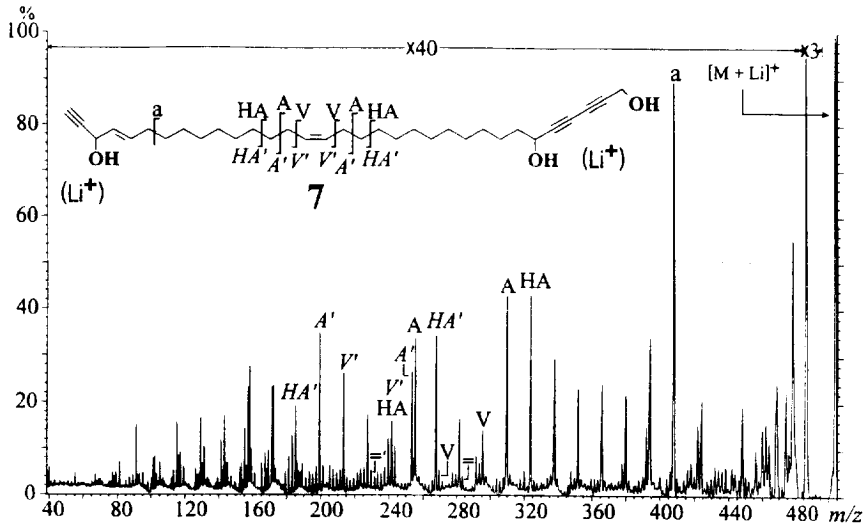


Figure 1. Four-sector tandem mass spectrum of compound **7** showing the charge-remote fragmentations. The C-C bond cleavages are denoted as V (vinylic), A (allylic), HA (homoallylic). Two series of charge-remote fragments are expected depending on the location of Li^+ (shown at one end or the other), and each series is denoted by a different type font.

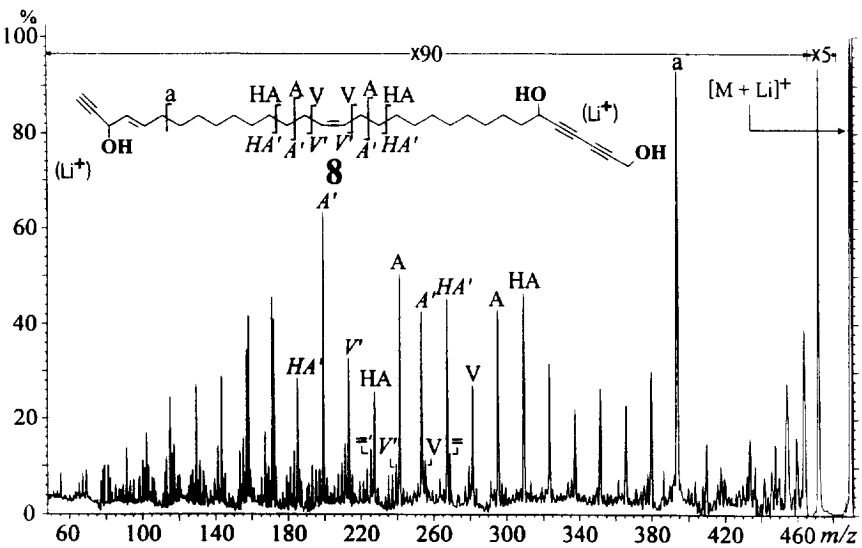


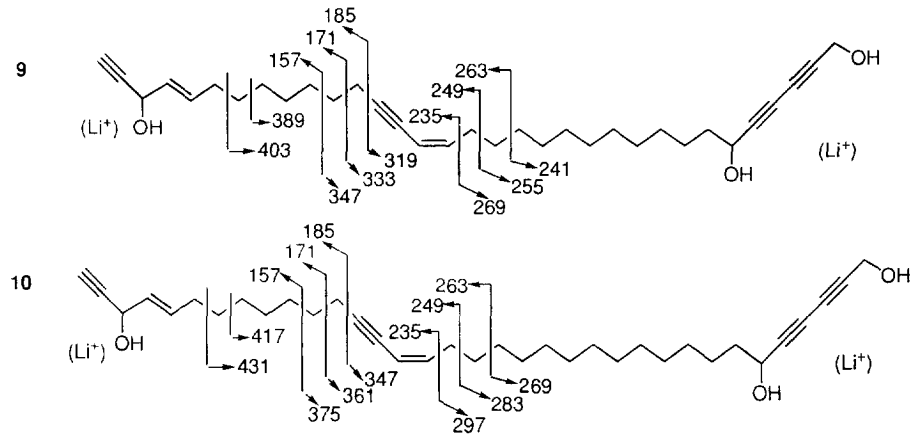
Figure 2. Four-sector tandem mass spectrum of compound **8** showing the charge-remote fragmentations. The C-C bond cleavages are denoted as V (vinylic), A (allylic), HA (homoallylic). Two series of charge-remote fragments are expected depending on the location of Li^+ (shown at one end or the other), and each series is denoted by a different type font.

Both of these series are consistent with the double bond located at C-18, as it is in **1**.

The tandem mass spectrum of **8** is also comprised of two sets of charge-remote fragment ion series (see Figure 2). The location of the double bond can be determined by a similar reasoning process as that used for **7**. When the charge is located on the diacetylenic side of **8**, the ion series is *m/z* 309 (HA), 295 (A), 281 (V), 255 (V), 241 (A), and 227 (HA). When the charge is on the monoacetylenic side, the series is *m/z* 267 (HA´), 253 (A´), 239 (V´), 213 (V´), 199 (A´), and 185 (HA´). The series are consistent with the double bond at C-17. It is interesting that the cleavages of the vinyl bond between C19 and C20 for **7** and C18 and C19 for **8** produce unexpectedly abundant product ions at *m/z* 295 and 213 for **7** and *m/z* 281 and 213 for **8**. This phenomena is not understood.

The tandem mass spectrum of **9** that is ionized by FAB also consists of two charge-remote fragmentation series that are superimposed on each other. The location of the enyne unit can be determined by an interpretation of these ion series which are shown in Scheme 3 and Table 3. The mass difference of 50 u between the two ions formed by vinyl bond cleavages is consistent with the enyne unit, and the *m/z* values of the series indicate that the enyne unit is at C-18. The relative positions of the double and triple bonds, however, cannot yet be determined simply from the tandem mass spectrum.

Scheme 3. CAD MS/MS Fragmentation Pattern for **9** and **10.**



The relative position of the enyne unit for **10** can also be determined from the tandem mass spectrum as is outlined in Scheme 3 and Table 3. The dual series of fragments are consistent with an enyne unit that is located at C-20, but the relative positions of the double and triple bonds in the enyne unit cannot be determined from the results of tandem mass spectrometry or chemical degradation.

The position of the ketone in **11** is difficult to fix on the basis of tandem mass spectrometry because again at least two series of charge-remote fragments are expected. Furthermore, the mass of the CO unit is the same as that of $-CH_2CH_2-$. Although no systematic studies of the charge-remote fragmentation of hydrocarbon chains bearing ketone groups have been published, we obtained CAD spectra of several ketone-containing fatty acids that were desorbed as $[M - H + 2Li]^+$ ions. There is a pattern, starting at low mass, of three abundant ions: a, a + 14, and a + 70. The first two ions are produced by charge-remote cleavages of the bond that is $\alpha\beta$ and the bond that is between the carbonyl and the α carbon, respectively; these bonds are on the side of the ketone that is more proximal to the charge site. The third ion of highest mass is formed by charge-remote

cleavage of the bond that is βγ on the side of the carbonyl that is more remote to the charge site. The relative abundances are high for the a and a + 70 and medium for the a + 14.

For an unknown with a charge site on either end of the molecule, two triplets would normally be expected. For the compound in question, the two triplets are at *m/z* 255, 269, 325 and 213, 227, 283 if the ketone is at C18. If the ketone is at C19, we expect triplets at *m/z* 269, 283, 339 and 199, 213, 269, which share the common *m/z* 269 fragment.

The observed pattern (see Table 3) fits reasonably well for a compound with the ketone at C19 except the abundance of the *m/z* 325 ion is unusually high. If the sample is a mixture of two components of comparable amounts having the ketone at C18 and C19, the CAD spectrum can be understood. The presence of a mixture is also consistent with the GC/MS results that were discussed earlier for this sample.

We can rule out any significant contributions of compounds having the ketone at C17 or C20. The observed spectrum also shows that abundant ions at *m/z* 423 and 409 are formed. These are, as expected, from allylic and homoallylic cleavages at the bonds joining C27/C28 and C26/C27, respectively.

BIOLOGICAL ACTIVITY

Pellynic acid (**1**) inhibited IMPDH with an IC_{50} of 1.03 μM. Pellynic acid methyl ester (**2**) and the acetylenic alcohols **7**-**11** were inactive.

EXPERIMENTAL SECTION

General Methods. All solvents were redistilled. Merck silica gel 60 (230-240 mesh) was used for vacuum flash chromatography; solid phase cartridges were J.T. Baker, 3 mL Bakerbond spe. HPLC was conducted by using a UV detector and Whatman Partisil 10 ODS and SPHEREX 5 C-18 columns. IR spectra were taken on a Bio-Rad 3240-SPC FT instrument, UV spectra on a Hewlet Packard spectrophotometer, and optical rotations on a Rudolph Autopol III automatic polarimeter at 589 nm. NMR experiments were conducted with Varian XL-300 and VXR-500 instruments; signals are reported in parts per million (δ), referenced to the solvent used. Normal EIMS and FABMS were measured on a Hewlett-Packard and VG ZAB-E mass spectrometers, respectively. The FAB tandem mass spectra were obtained with a VG ZAB-T (Manchester, UK) four-sector tandem mass spectrometer.[28] The samples were spotted onto the FAB probe that held a matrix of 3-nitrobenzyl alcohol (Aldrich, St. Louis, Mo) that was saturated with LiI (Aldrich, St. Louis, Mo). Desorption was by a Cs ion gun that was operated at an acceleration potential of 25 kV. The parent-ion beam was accelerated to 8000 eV, and, for most experiments, was delivered to the collision cell after the first stage mass spectrometer at a mass resolving power of 1000. The collision cell was floated at 4000 V, providing a laboratory collision energy of 4000 eV. Helium gas was admitted to the collision cell to a pressure sufficient to suppress the main ion beam to 30%. The second-stage mass spectrometer scan was calibrated by using a cluster ion of *m/z* 553 of glycerol (Aldrich, St. Louis, Mo) that was desorbed by Cs^+ bombardment from a FAB probe in the ion source. Detection was with a single point detector.

For compound **1**, the beam of parent ions was of such low intensity that the resolving power of the first stage mass spectrometer was increased to 2000 to eliminate interference by matrix ions. Furthermore, to improve signal-to-noise, the product ions were detected by using a 4096-point extended array detector that was made of two 2048 arrays that were joined in the center, leaving a 20-element gap in the center. The array was at 15° to the main beam, allowing for detection of a mass range of 1.22:1.00. The second-stage mass spectrometer scan was calibrated by using various cluster ions of CsI that were desorbed by Cs^+ bombardment of solid CsI

on a solids probe located in the ion-source region between the two mass spectrometers. A product-ion spectrum was obtained by joining together 11 exposures on the array. For all tandem spectra, approximately ten scans were averaged to give the final mass spectrum.

Animal Material. Sponge specimens were collected from underneath overhangs and in caves at depths of 20-30 m, from Chuuk Atoll, Federated States of Micronesia, on June 2, 1992 and August 8, 1993. The sponge forms fist-sized hemispherical cushions 2-4 cm thick with occasional slender and fragile fistules arising from the sponge surface. The sponge has several large oscules with raised rims, and the surface is smooth, papery and detachable, the texture of the interior crumbley. The color in life is white with a pale pink tinge and beige internally. In ethanol, the sponge is peach-orange. Megascleres and small slim oxea are arranged in an isotropic reticulation, or are completely confused in orientation, the surface is a crust of tangential spicules. Spicule tracts are discontinuous and rare. The sponge is most closely comparable to *Pellina triangulata* Desqueyroux-Faundez 1987 (Order Petrosida, Family Oceanapiidae). A voucher specimen has been deposited at The Natural History Museum, London (BMNH:1996:8:6:1)

Extraction and Isolation. Freshly thawed sponge, *Pellina triangulata* (8-T-92), (1.5 kg wet wt., 150 gm dry wt. after extraction) was extracted twice with MeOH overnight, and then twice with CH_2Cl_2 overnight. Both MeOH and CH_2Cl_2 extracts exhibited activity against IMPDH with IC_{50} values of 2.9 and 1.6 μg/mL, respectively. The extracts were combined, dissolved in 10% aq. MeOH, and this solution was extracted with hexane. The aqueous solution was diluted with H_2O to 30% H_2O in MeOH and extracted with $CHCl_3$, and, after most of the MeOH had been evaporated, extracted also with n-BuOH. The $CHCl_3$ phase was concentrated to dryness to give a residue (IMPDH IC_{50} = 1.2 μg/mL) which was subjected to silica gel chromatography using gradient elution (Hexane -> EtOAc -> MeOH). Twelve fractions were collected. Further chromatography of a major IMPDH active fraction (accounting for approximately 10% of $CHCl_3$ solubles) on an open SiO_2 column using the same elution system, followed by separation on a reverse phase HPLC column (Whatman Partisil 10 ODS) using H_2O-MeOH (9:41) as eluent gave pure pellynic acid (**1**) as a gum. A portion of one of the less polar IMPDH inactive fractions from the first SiO_2 column was resolved by reverse phase C-18 HPLC (Spherex 5, 300 x 10 mm) by using H_2O-MeOH (3:17) as eluent and yielded three new acetylenic compounds **7** (53.4 mg), **9** (49.5 mg), and **11** (20.2 mg), and a mixture fraction. This mixture was further purified by reverse phase C-18 HPLC using H_2O-CH_3CN (1:9) as eluent to afford compounds **8** and **10**. Percent yields were not calculated because not all of the fractions were exhaustively processed.

Pellynic acid (**1**): $[\alpha]_D$ = -10.5° (c 0.34, $CHCl_3$/MeOH 1:1); LRFABMS (positive) *m/z* 535 $[M + K]^+$; LRFABMS (negative) *m/z* 495 $[M - H]^+$; HRFABMS *m/z* 535.3553 (calc'd for $C_{33}H_{52}O_3K$, 535.3554) $[M + K]^+$; IR (neat) (salt form) ν_{max} 3560, 3290, 2209, 1585, 1550, 1405, 965 cm^{-1}; (acid form) 1686 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1.

Pellynol A (**7**): $[\alpha]_D$ = -8.5° (c 1.0, $CHCl_3$); HRFABMS *m/z* 519.3787 $[M + Na]^+$ calc'd for $C_{33}H_{52}O_3Na$, 519.3814); IR (neat) ν_{max} 3200-3500 (br), 2250, 2110, 1645, 1465, 1020, 970 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2.

Pellynol B (**8**): $[\alpha]_D$ = -7.6° (c 0.28, $CHCl_3$); IR, ^1H and ^{13}C NMR data are virtually identical to those of

pellynol A; LRFABMS (3NBA matrix) *m/z* 505 $[M + Na]^+$; HRFABMS *m/z* 505.3644 (calc'd for $C_{32}H_{50}O_3Na$ 505.3658).

Pellynol C (**9**): $[\alpha]_D$ = -11.2° (c 2.38, $CHCl_3$); IR (neat) cm^{-1} 3300 (br), 2250, 2200, 2160, 2115, 1670, 1610, 1454, 1015, 965 cm^{-1}; HRFABMS *m/z* 515.3496 $[M + Na]^+$ (calc'd for $C_{33}H_{48}O_3Na$ 515.3501); LRFABMS *m/z* 499 $[M + Li]^+$; ^1H and ^{13}C NMR data, see Table 2.

Pellynol D (**10**): $[\alpha]_D$ = -9.8° (c 0.64, $CHCl_3$); IR, ^1H and ^{13}C NMR data are virtually identical to those of pellynol C; LRFABMS (3 NBA matrix) *m/z* 543 $[M + Na]^+$ HRFABMS *m/z* 543.3800 (calc'd for $C_{35}H_{52}O_3Na$ 543.3814).

Pellynone (**11**): $[\alpha]_D$ = -9.0° (c 0.45, $CHCl_3$); IR (neat) λ_{max} 3400 (br), 2240, 2120, 1712, 1630, 1470, 1410, 1020 cm^{-1}; LRFABMS *m/z* 535 $[M + Na]^+$; LRFABMS *m/z* 519 $[M + Li]^+$; HRFABMS *m/z* 535.3731 $[M + Na]^+$ (calc'd for $C_{33}H_{52}O_4Na$, 535.3763); ^1H and ^{13}C NMR data, see Table 2.

Methylation of pellynic acid (**1**). (a) One-half mL of Et_2O containing CH_2N_2 was added to pellynic acid (2 mg) and after standing 5 min. at room temperature the solution was evaporated to dryness under reduced pressure to give methyl ester **2** (2.1 mg), $[\alpha]_D$ = -9.3° (c 0.1, $CHCl_3$); IR (neat) λ_{max} 3400, 2207, 2115, 1700 (vs), 1610, 1450, 1430, 1260 cm^{-1}; EIMS (12 eV) *m/z* (rel. int. %) 479 (2.3) $[M - OCH_3]^+$, 451 (6.5) $[M - COOCH_3]^+$, 433 (31.9) $[M - H_2O - COOCH_3]^+$, 95 (100), 81 (73.9); ^1H and ^{13}C NMR data, see Table 1.

(b) A solution of pellynic acid (~5 mg), 0.2 mL of 12% w/w boron trifluoride-methanol reagent[22] and an excess of dry methanol (3 mL) was stirred and refluxed under nitrogen for 12 hrs. The methanol was removed *in vacuo*, and the resulting mixture was partitioned between H_2O (2.5 mL) and CH_2Cl_2 (2.5 mL). The CH_2Cl_2 layer, after removal of solvent, was subjected to chromatography over a 3 mL SiO_2 cartridge (Bakerbond spe) using CH_2Cl_2/hexane (1:1) as eluent to afford compound **3** (3.8 mg) which was also prepared by the following procedure.

(c) Chlorotrimethylsilane (50 μL) was added to a solution of pellynic acid (~5 mg) in dry methanol (2 mL) under a nitrogen atmosphere. The mixture was stirred at room temperature for 12 hrs. The methanol was removed under reduced pressure, and the residue was passed through a 3 mL SiO_2 cartridge using CH_2Cl_2/hexane (1:1) as eluent to yield **3** (3.5 mg), $[\alpha]_D$ = -12.1 (c 0.31, $CHCl_3$); EIMS (12 eV) *m/z* 465 $[M - COOCH_3]^+$; FABMS *m/z* 493 $[M - OCH_3]^+$; ^1H and ^{13}C NMR, see Table 1.

(d) A mixture of **1** (8.5 mg) and 3 mL of CH_2N_2 in Et_2O was kept at room temperature overnight. The residue, after removal of solvent, was subjected to reverse phase HPLC using MeOH as eluent to furnish compounds **4**, **5**, and **6** in a ratio of 3:4:2, respectively, as estimated by the integration of the ^1H NMR spectrum of the reaction mixture.

Compound 4: $[\alpha]_D$ = -11.2° (c 0.36, $CHCl_3$); IR (neat) λ_{max} 3400 (br), 2216, 1719 (vs), 1650, 1536, 1460, 1256, 1100 cm^{-1}; EIMS (12 eV) *m/z* 566 $[M]^+$, 548 $[M - H_2O]^+$; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.77 (dt, *J* = 11.6, 1.2 Hz, 1H, H-4); 5.84 (dt, *J* = 11.6, 7.3 Hz, 1H, H-5), 2.55 (dq, *J* = 1.2, 7.3 Hz, 2H, H-6), 2.00 (dt, *J* = 6.0, 7.0 Hz, 4H, H-17, 20), 5.34 (t, *J* = 4.9 Hz, 2H, H-18, 19), 2.06 (q, *J* = 6.5 Hz, 2H, H-28), 5.91 (dt, *J* = 15.3, 6.5 Hz, 1H, H-29) 5.60 (ddd, *J* = 15.3, 6.1, 1.2 Hz, 1H, H-30), 4.83 (brd, *J* =

6.1 Hz, 1H, H-31), 2.56 (d, *J* = 2.4 Hz, 1H, H-33), 7.87 (s, 1H, H-34), 3.80 (s, 3H, OCH_3), 3.90 (s, 3H, N-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 163.8 (C-1), 112.2 (C-2), 134.1 (C-3), 118.0 (C-4), 137.0 (C-5), 27.2 (C-17,20), 129.9 (C-18,19), 31.9 (C-28), 134.6 (C-29), 128.3 (C-30), 62.8 (C-31), 83.3 (C-32), 73.9 (C-33), 150.5 (C-34), 51.1 (OCH_3), 39.4 (N-CH_3).

Compound 5: $[\alpha]_D$ = -11.8° (c 0.40, $CHCl_3$); IR (neat) λ_{max} 3400, 1717 (vs), 1651, 1460, 1260, 1115, 1015 cm^{-1}; EIMS (12 eV) *m/z* 566 $[M]^+$, 548 $[M - H_2O]^+$; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.55 (brd, *J* = 11.4 Hz, 1H, H-4); 5.68 (dt, *J* = 11.4, 6.6 Hz, 1H, H-5), 2.27 (dq, *J* = 1.4, 7.1 Hz, 2H, H-6), 2.00 (dt, *J* = 5.7, 7.0 Hz, 4H, H-17, 20), 5.34 (t, *J* = 5.2 Hz, 2H, H-18, 19), 2.06 (q, *J* = 7.1 Hz, 2H, H-28), 5.91 (dt, *J* = 16.6, 6.6 Hz, 1H, H-29) 5.60 (dd, *J* = 16.6, 6.2 Hz, 1H, H-30), 4.83 (brd, *J* = 6.2 Hz, 1H, H-31), 2.55 (d, *J* = 2.4 Hz, 1H, H-33), 7.52 (s, 1H, H-34), 3.91 (s, 3H, OCH_3), 4.15 (s, 3H, N-CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 160.9 (C-1), 123.4 (C-2), 115.2 (C-3), 118.5 (C-4), 137.90 (C-5), 28.82 (C-6), 27.2 (C-17,20), 129.9 (C-18), 129.86 (C-19), 31.93 (C-28), 134.6 (C-29), 128.3 (C-30), 62.8 (C-31), 83.4 (C-32), 73.9 (C-33), 133.8 (C-34), 51.9 (OCH_3), 40.3 (N-CH_3).

Compound 6: $[\alpha]_D$ = -10.5° (C 0.18, $CHCl_3$); EIMS (12 eV) *m/z* 566 $[M]^+$, 548 $[M - H_2O]^+$, 533 $[M - H_2O - Me]^+$; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.25 (br d, *J* = 11.6 Hz, 1H, H-4), 6.04 (dt, *J* = 11.6, 7.3 Hz, 1H, H-5), 1.92 (dq, *J* = 1.2, 7.3 Hz, 2H, H-6), 2.00 (dt, *J* = 5.5, 7.3 Hz, 4H, H-17, 20), 5.34 (t, *J* = 4.9 Hz, 2H, H-18, 19), 2.06 (q, *J* = 7.3 Hz, 2H, H-28), 5.91 (dt, *J* = 15.3, 7.3 Hz, 1H, H-29), 5.60 (dd, *J* = 15.3, 6.1 Hz, 1H, H-30), 4.83 (br d, *J* = 6.1 Hz, 1H, H-31), 2.55 (d, *J* = 2.4 Hz, 1H, H-33), 7.89 (s, 1H, H-34), 3.78 (s, 3H, N-CH_3), 3.73 (s, 3H, OCH_3).

Typical procedure for degradation of acetylenic compounds. Ozone was passed through a stirred solution of the acetylenic compound in CH_2Cl_2 at -78°C until the color of the solution turned pale blue. After removal of excess O_3 in the solution by passage of N_2, the reaction flask was removed from the cooling bath and allowed to reach room temperature. The reaction solution was evaporated by a stream of N_2. Glacial acetic acid and 30% H_2O_2 were added to the reaction residue and the resulting mixture kept at rt overnight (14 hrs.). The products were dried with a stream of N_2, dissolved in MeOH, and then treated with CH_2N_2 in diethyl ether. After the solvent was removed by a stream of N_2, the products were submitted to GC-MS analysis.

Enzyme Assay. The inosine monophosphate dehydrogenase bioassay was conducted as described previously[3] with dithiothreitol added and with enzyme added last.

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