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Structures and Cytotoxic Evaluation of New and Known Acyclic Ene-Ynes from an American Samoa *Petrosia* sp. Sponge

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Abstract

Four new compounds, (–)-petrosynoic acids A–D (**1–4**), and five known congeners, pellynols A (**5**), C (**6**), D (**7**), F (**8**), and I (**9**) were isolated from a *Petrosia* sp. marine sponge collected in American Samoa. Isolation work was guided by cytotoxicity against human lung cancer cells (H460). The structures of the C₃₁–C₃₃ polyacetylenes (**1–9**) were determined on the basis of 1D- and 2D-NMR analysis, mass spectrometry, and comparison of specific rotation values. Compounds **1–9** were found to be broadly cytotoxic with limited selectivity for cancer cells as they were all moderately active against the A2058 (melanoma), H522-T1 (lung), and H460 (lung) human cancer cell lines as well as IMR-90 quiescent human fibroblast cells.

Several years ago we concluded that marine sponges from American Samoa are an attractive target for further systematic chemical study. Our search of the natural history literature pertaining to sponges from Samoa indicated a reasonable biodiversity of taxa observed in several ecological niches.¹ By contrast, our current awareness searches revealed only four papers published on biosynthetic products isolated from Samoan sponges.^{2–5} The list of compound types reported from this group is rather small but does include diverse classes such as: (a) a ubiquitous PKS/NRPS macrolide, latrunculin A,² a dual cytotoxic actin inhibitor;⁶ (b) the PKS macrocycle, fijianolide A (a.k.a. isolaulimalide),² a potent microtubule stabilizing agent;⁷ (c) the shikimate-sesquiterpene, puupehenone,³ active against *M. tuberculosis*⁸ and human lipoxogenases;⁹ (d) the common scalarane sesterterpene, heteronemin,⁴ active against *M. tuberculosis*⁸ and showing inhibition of TNF- α induced NF- κ B activation;¹⁰ and (e) the oroidin alkaloid, palau'amine,⁵ a cytotoxic and immunosuppressive agent that has been shown to inhibit the human 20S proteasome.^{11,12}

We have amassed a collection of 23 distinct sponges from several shallow water sites in the American Samoa region. The accelerated solvent extracts (ASE) of each were screened for activity against cancerous human cell lines, and one registered as a potent hit, completely

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DEDICATION

Dedicated to Dr. Lester A Mitscher, of the University of Kansas, for his pioneering work on the discovery of bioactive natural products and their derivatives.

ASSOCIATED CONTENT

Supporting Information

Isolation scheme, ¹H and ¹³C NMR spectra for extract 10018 XFM and compounds **1–9**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

inhibiting the growth of H460 human cancer cells (lung) at 10 $\mu\text{g/mL}$ concentration. Thus, the methanol-soluble extract of a *Petrosia* sp. (order Haplosclerida, UCSC Coll. No.10018, extract coded XFM) was selected for detailed chemical study. Sponges of this genera have been linked to broad activities including antifungal, antiviral and anti-inflammatory effects, however, they are also a major contributor to the polyacetylene class of cytotoxic sponge-derived secondary metabolites.¹³ Thus far, there have been no studies on the secondary metabolites of *Petrosia* sp. collected from Samoan waters. We now describe the isolation, structure determination and bioactivity assessment of four new linear polyacetylenes, (–)-petrosynoic acids A–D (**1–4**) (Figure 1), and five known congeners, pellynols A (**5**), C (**6**), D (**7**), F (**8**) and I (**9**) (Figure 2).

RESULTS AND DISCUSSION

Samples of *Petrosia* sp. were collected from America Samoa and immediately preserved and stored according to our standard laboratory procedures.¹⁴ A portion of the specimen (11.1 g) was worked up using a recently validated accelerated solvent extraction (ASE) protocol.¹⁵ Four crude solvent extract fractions were obtained and included a water wash (extract code XFW: 2.3 g), followed by organic solvent fractions of: hexanes (extract code XFH: 0.025 g), dichloromethane (extract code XFD: 0.3 g) and methanol (extract code XFM: 1.5 g) (Scheme S1; S denotes Supporting Information). Since the XFM extract displayed significant in vitro cytotoxicity (100% inhibition of H460 cell line at 10 $\mu\text{g/mL}$) it was prioritized for immediate RP-LC profiling by parallel PDA-ELSD-MS analysis. Several late-eluting compounds were visualized in the ELSD trace obtained from the polar XFM fraction, as shown in Figure 3, which was a rather surprising outcome.

The dereplication efforts involved a three-pronged approach: MS analysis, biological source searches, and NMR analysis. Initial attempts to correlate MS m/z ions with molecular mass queries were thwarted due to ambiguous ionization data. Insights from the taxonomic identification were useful; a recent book chapter on sponge-derived polyacetylenic compounds highlighted that the genus *Petrosia* is a source of the majority of such metabolites.¹³ Next, parallel inspection the ^1H and ^{13}C NMR data obtained on the XFM fraction, shown in Figure 4, was productive because the major peaks observed could be correlated to several alkyne and alkene substructures, as will discussed below. At this stage, our provisional conclusion was that most or all of the peaks of Figure 3 could be variants of polyacetylene/unsaturated fatty-acid chemotypes. Directly supporting this supposition were the data of Figure 4 in which the following were observed: six ^{13}C resonances consistent with sp^2 hybridization, as observed for other *Petrosia* compounds, between δ_{C} 68–85, and four sp^2 ^1H resonances between δ_{H} 5.35–6.20.¹⁶ Further analysis indicated that many of the peaks of Figure 4 could be correlated to residues present in two known compounds, melynene A¹⁷ and pellynol A.¹⁸ Semi-preparative RP-HPLC chromatography afforded seven fractions (coded H1–H7, Scheme S1) that were the source of the nine polyacetylenes. As discussed next, we determined that the major metabolite was actually the latter and that the former was not present in the mixture.

The decision to first isolate the constituent(s) of the major peak with $R_t = 20.5$ min (Figure 3, also coded as H5 (105 mg) in Scheme S1) proved to be beneficial and a subsequent round of RP-HPLC afforded two pure compounds, **5** (66.0 mg) and **1** (4.1 mg). Molecular formulas of $\text{C}_{33}\text{H}_{52}\text{O}_3$ (UN = 8) and $\text{C}_{31}\text{H}_{48}\text{O}_3$ (UN = 8) were established for the major component **5** and the minor component **1**, respectively (HRESITOFMS). Using the formula of **5** as input for dereplication searches, four linear polyacetylene hits were generated that could be divided into two sets of acyclic isomers (Figure S1). All four compounds were capped on one end with an identical yne-ol terminus, but they differed by the presence at the other end of either a conjugated ene-yne-acid residue (triangulynic acid¹⁹ and pellynic

acid¹⁸) or a conjugated diyne-diol moiety (melyne A¹⁷ and pellynol A¹⁸). The ¹³C NMR spectrum of **5** displayed six sp carbon resonances (annotated in Figure 4 for the XFM crude extract) thus ruling out the triangulynic acid and pellynic acid possibilities. In differentiating between melyne A and pellynol A, we opted to place the internal double bond by means of EIMS analysis of TMS derivatized products (**5**, *m/z* 712, C₄₂H₇₆O₃Si₃) as previously reported for similar polyacetylenes.^{20,21} Definitive HRMS fragmentation ions (Figure S3) were observed and we thus finalized the planar structure assignment as that of pellynol A (**5**). This result provided a benchmark to assign the structures of three analogues that were isolated next. The levorotatory specific rotation of compound **5** matched the literature value of pellynol A, however, we did not pursue the assignment of the absolute configuration of this known compound.

Further HPLC purification of the remaining fractions eventually led to the isolation of additional known pellynols (Figure 2), which were quickly characterized by the intense ¹H NMR resonance at δ 4.24 (see Figure 4). These were as follows: pellynol C (**6**, 7.0 mg)¹⁸ as the major component of fraction H1, pellynol D (**7**, 3.9 mg)¹⁸ as a component of H2, pellynol F (**8**, 1.5 mg)²² as a minor component of H3, and pellynol I (**9**, 64 mg)²³ as nearly the sole component of H6. Compounds **6** and **7** were identified as described above for pellynol A (**5**) using NMR data and specific rotation measurements in addition to EIMS fragmentation of TMS derivatization products. The data for compounds **8** and **9** matched pellynol F and pellynol I, respectively, except the EIMS analysis of their TMS derivatization products was ambiguous. Consequently, our assignment of the alkyne of **8** was provisional and based on biosynthetic analogy to previous isolation work, and it should be noted that no investigator firmly located the position of the branching methyl in **9**.

(–)-Petrosynoic acid A (**1**) was isolated as a minor component from the H5 fraction of the XFM extract (Scheme S1). Dereplication searches on sponge metabolites with its formula, C₃₁H₄₈O₃ (HRESITOFMS, UN = 8), yielded no hits. We did however recognize substructures **A** and **C** in the ¹H and ¹³C NMR spectra (Figure S4), and were able to assign terminal substructure **B** based on key COSY and HMBC correlations shown in Figure 5. The ³*J* value of 15.3 Hz confirmed the *E* double bond geometry of **A** and the diagnostic allylic δ_C 28.3 confirmed the *Z* double bond geometry of **B**.¹⁶ An observed loss of -CO₂H (MS² *m/z* 423.9) from the parent ion (*m/z* 467.3) in a complementary tandem MS scan of **1** confirmed the carboxylic acid assignment. The ensemble of functionalities in **1** matched those found in triangulynic acid¹⁹ and pellynic acid¹⁸ (Figure S1), but with a C₂H₄ residue removed from the formula. The *bis*-TMS derivatization product of **1** was analyzed by EIMS (*m/z* 612, C₃₇H₆₄O₃Si₂) and the diagnostic high-resolution fragment ions annotated in Figure 6 justified the double bond placement between C-15 and C-16. The 3*R* absolute configuration of **1** was provisionally assigned based on a correlation pattern of specific rotation values in the following *R* series of analogous compounds (see Figure S8): (a) (–)-triangulynic acid,¹⁹ and (b) (–)-corticatic acids A, D and E,²⁴ all of which were assigned using the advanced Mosher method.

Completion of the structure elucidation of **1** greatly simplified the characterization of compounds **2** and **3**. The constitutions of (–)-petrosynoic acid B (**2**), C₃₃H₅₀O₃ (HRESITOFMS, UN = 9), and (–)-petrosynoic acid C (**3**), C₃₃H₄₈O₃ (HRESITOFMS, UN = 10), were isomeric to pellynol F (**8**) and pellynol C (**6**), respectively. It was evident from the ¹H NMR data that compounds **2** and **3** (Figure S5 and Figure S6) shared the terminal substructures **A** and **B** assigned in **1** (Figure 5). Additionally, we were able to assign substructure **D** in **2** and substructure **E** in **3** based on the COSY and HMBC correlations annotated in Figure 5. The placement of a C-15/C-16 alkyne in **2** and a C-13/C-16 ene-yne in **3** was justified by the HREIMS fragmentation profiles of their respective TMS products (**2**, *m/z* 638, C₃₉H₆₆O₃Si₂; **3**, *m/z* 636, C₃₉H₆₄O₃Si₂) shown in Figure 6. An exact

regiochemical assignment of substructure **E** in **3** (i.e., from left to right the yne-ene could be reversed to ene-yne) was not pursued. Absolute configurations of 3*R* were provisionally assigned to compounds **2** and **3** based on the correlation of levorotatory specific rotation as described for **1**.

The final compound examined was (–)-petrosynoic acid **D** (**4**), C₃₃H₅₂O₄ (HRESITOFMS, UN = 8), a constitutional isomer of the sponge-derived polyacetylenes pellynone¹⁸ and pellynol G²² (Figure S9). All signature NMR resonances, except for that of C-33, were observed to identify substructures **A** and **B** (Figure S7). Evidence to further justify the terminal carboxylic acid functionality came from a fragment ion observed at *m/z* 467.8 [M-CO₂H][–] during a tandem MS scan. The final element of unsaturation was rationalized by proposing substructure **F**, which was defined by the NMR data shown for positions #16–20 in Table 1 along with the COSY and HMBC correlations sketched in Figure 5. Embedding the allylic alcohol group within the alkyl chain was crisply addressed by EIMS analysis of the *tris*-TMS derivatization product of **4** (*m/z* 728, C₄₂H₇₆O₄Si₃), and the diagnostic high-resolution ion at *m/z* 333.2612 (C₂₁H₃₇OSi) annotated in Figure 6. Finally, by analogy to arguments presented above, the 3*R* configuration was provisionally assigned based on the observed levorotatory specific rotation.

As this study progressed, we fully recognized that the antitumor therapeutic potential of sponge-derived polyacetylenes, especially those containing the 1-yne-3-ol array (substructure **A**), has been extensively examined. Furthermore, highlights about the therapeutic lead potential of such structures have been discussed in recent reviews,^{13,25} including the outcome that one compound (see **I** in Figure S10) exhibited a small in vivo T/C = 130% against IGROV-1 (human ovarian) cancer cells in athymic mice.²⁶ Based on previous findings (see structures **II**,²⁷ **IV**,²⁶ **VI**¹⁹ and **VIII**,²⁸ Figure S10), compounds containing substructure **A**, present in all of our isolates (**1–9**), would be expected to exhibit low to sub-micromolar in vitro cytotoxicity to a range of solid tumor cancer cells. Unknown from the literature were the responses to be expected for these compounds against non-proliferating normal cells.

An in vitro cytotoxicity assay was employed, using the panel of cell lines shown in Table 2, to distinguish between true antiproliferative activity and general cellular cytotoxicity unrelated to proliferation. This general strategy, as previously described,²⁹ consisted of the following cell types: one non-proliferating human fibroblast, IMR-90, and three human tumor types, A2058 (melanoma), H522-T1 (lung), and H460 (lung). The specificity in responses was measured by calculating an IC₅₀ value selectivity index (SI) based on the relative response between IMR-90 and A2058 lines. All nine compounds were active against each of the tumor cell lines with IC₅₀ values ranging from 0.4 to 2.7 μM; but all compounds were also similarly potent against the IMR-90 cells. The benchmark for selective cytotoxicity was observed for the anticancer drug vinblastine, which displayed an SI_{A2058} greater than 250. By contrast, none of the polyacetylenes demonstrated comparable selectivity, as the best response was observed for pellynol **F** (**8**), for which the SI_{A2058} was 1.2. Thus, these compounds do not have an in vitro therapeutic window between proliferating and quiescent cells, indicating that the cell growth inhibitory effects observed against human cancer cells result from indiscriminant proliferation-independent cytotoxicity, rather than from inhibiting proliferative processes.

The pellynols (**5–9**) were dominant in the crude extract vs. the (–)-petrosynoic acids (**1–4**) as the overall isolated yield ratio was 10:1 (see Figure 3 and Scheme S1). Interestingly, a similar trend was observed by Schmitz in the rough relative isolation yield ratios of pellynols (shown in Figure 3) vs. pellynic acid (see structure **X**, Figure S10) from *Pellina triangulata*.¹⁸ Perhaps it is the polyacetylene acids (pellynic and petrosynoic) that are the

more interesting, because the former, unlike their corresponding triols, are selectively active against the cancer target inosine monophosphate dehydrogenase (IMPDH).¹⁸ It is also tempting to suggest that the acids are a biosynthetic product of the triols because of their overall smaller relative isolated yields.

The nine acyclic polyacetylene compounds isolated here join sponge-derived bioactive congeners that been under active study since their first report in 1977.³⁰ Astoundingly, this class has expanded to greater than 270 members, with roughly 33% isolated from the marine sponge genera *Petrosia* (order Haplosclerida).¹³ At this juncture the prospects for further therapeutic lead development of the active pharmacophore associated with substructure **A** continues to be a challenge. The major issues include: (a) potent but indiscriminant cytotoxicity, demonstrated above, accompanied by broad toxicity to mammalian cells, (b) contradictory patterns of structure-related cytotoxicity (as shown by three sets of entries in Figure S10: see **II** vs. **III**,²⁷ **IV** vs. **V**,²⁶ and **VIII** vs. **IX**²⁸), (c) general compound instability, and (d) incomplete understanding about the molecular target associated with their cytotoxicity.¹³

EXPERIMENTAL SECTION

General Experimental Procedures

Specific rotations were measured using a JASCO P-2000 polarimeter. Standard pulse sequences were used for all NMR experiments, which were run on a Varian UNITY INOVA spectrometer (600 and 150 MHz for ¹H and ¹³C, respectively) equipped with a 5 mm triple resonance (HCN) cold probe. Residual solvent shifts for methanol-*d*₄ were referenced to δ_H 3.31 and δ_C 49.15, respectively. Accurate mass measurements were obtained on a Mariner ESITOF instrument for molecular formula determinations. Low-resolution tandem mass spectrometry was performed on a Thermo Deca ESI-ion trap instrument. Electron-impact mass spectrometry experiments were conducted on a Auto Spec Premier instrument (Waters, Manchester, UK; ion source set at 250°C) with direct sample introduction. Accelerated solvent extraction (ASE) was performed using a Dionex model 100 apparatus. All solvents used for ASE extractions were HPLC grade and included water, hexanes, dichloromethane and methanol. All chromatographic work was reversed-phase and utilized HPLC grade CH₃CN and Milli-Q H₂O, both adjusted to contain 0.1% formic acid. The analytical LC-UV-ELSD-MS system was controlled by Empower software and comprised of Waters HPLC components (i.e., solvent pumps and auto-sampler) equipped with a 150 × 4.60 mm 5 μ m Luna C₁₈ column (Phenomenex) and operated at a flow rate of 1 mL/min. The post-column flow of the eluent was first through a photo-diode array (Waters) and then split (1:1) between an evaporative light-scattering detector (SEDEX model 55) and a low-resolution electrospray ionization time-of-flight (ESITOF) mass spectrometer (Applied Biosystems Mariner). The semi-preparative HPLC system was comprised of Waters HPLC components (i.e., solvent pumps and gradient controller) and equipped with either a Luna C₁₈ or Synergi-Hydro RP 250 × 10 mm 5 μ m column (Phenomenex) and tunable UV-absorbance detector (Waters).

Animal Material

The *Petrosia* sp. (UCSC coll. no. 10018, 0.6 kg wet weight) was collected by SCUBA in July 2010 from Tutuila, American Samoa (S 14°16.60', W 170°36.72') at depths ranging from 15 – 30 m. The voucher fragment examined for taxonomy consisted of a small smooth globular branch with a detachable crust and several slightly elevated oscules. The ectosome formed a distinct rind and was easily detachable from the subdermal region. The ectosomal skeleton formed a loose reticulation of paucispicular tracts forming irregular meshes with many loose spicules in between the meshes. The choanosomal skeleton was a dense mass of

spicules in two distinct size classes laying in confusion. The larger spicules consisted of bent oxeas, stronglyloxeas and styles with irregular lumpy ends and a size range of $145\text{--}250 \times 2.5\text{--}5.0 \mu\text{m}$. The smaller spicules were sharply angled oxeas with a size range of $55\text{--}75 \times 1.5\text{--}2.5 \mu\text{m}$. The voucher is deposited at the Netherlands Centre for Biodiversity Naturalis, Leiden, under code number RMNH POR 4000.

Extraction and Isolation

Sponge samples were preserved in the field according to our standard laboratory procedures,¹⁴ transported back to the UCSC lab at ambient temperature and stored at 4°C until extraction was performed. ASE extraction was performed on 11.1 g dry, lightly chopped sponge at high pressure and temperature (1500 psi N_2 , 70°C) using the solvent series water (XFW: 2.3 g), hexanes (XFH: 25 mg), dichloromethane (XFD: 0.3 g), and methanol (XFM: 1.5 g). During each ASE extraction cycle the sponge material was three times exposed to approximately 200 mL of solvent for 30 min, similar to our previously validated method.¹⁵ The cytotoxic XFM extract was fractionated into seven fractions (stock solution: 825 mg/8 mL methanol, 76×0.1 mL injections, fraction codes H1–H7, Scheme S1) using an HPLC system equipped with a 250×10 mm $5 \mu\text{m}$ Synergi Hydro-RP column (Phenomenex), utilizing a flow rate of 2 mL/min, 254 nm detection and the following elution conditions: 5 min isocratic 90% CH_3CN in H_2O , 13 min gradient from 90% to 97% CH_3CN in H_2O , and 42 min at 100% CH_3CN . Fraction H1 (stock solution: 23.8 mg/0.1 mL methanol, 6×0.01 mL injections) was purified on an HPLC system equipped with a 250×10 mm $5 \mu\text{m}$ Luna C_{18} column (Phenomenex), utilizing a flow rate of 3 mL/min at isocratic conditions of 85% CH_3CN in H_2O and 254 nm detection, to yield **6** (7.0 mg) and **4** (1.4 mg). Fraction H2 (stock solution: 14.7 mg/0.1 mL methanol, 6×0.01 mL injections) was purified on the same HPLC system under isocratic conditions of 90% CH_3CN in H_2O with a flow rate of 3 mL/min and 230 nm detection, to yield **7** (3.9 mg) and **3** (2.6 mg). Fraction H3 (stock solution: 21 mg/0.1 mL methanol, 7×0.01 mL injections) was purified on the same HPLC system under isocratic conditions of 93% CH_3CN in H_2O with a flow rate of 3 mL/min and 230 nm detection, to yield **8** (1.5 mg) and **2** (4.6 mg). Fraction H5 (stock solution: 103 mg/0.2 mL methanol, $4 \times 50 \mu\text{L}$ injections) was purified on the same HPLC system under isocratic conditions of 95% CH_3CN in H_2O with a flow rate of 3 mL/min and 254 nm detection, to afford **5** (66 mg) and **1** (4.1 mg). Fraction H6 contained 64 mg of pure **9**.

(–)-*Petrosynoic acid A (1)*: clear oil; $[\alpha]_{\text{D}}^{24} -11.2$ (c 1.0, CHCl_3); ^1H NMR (600 MHz, methanol- d_4) and ^{13}C NMR (150 MHz, methanol- d_4) data, see Table 1 and Figure S4; ESIMS m/z 491.3483 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{31}\text{H}_{48}\text{O}_3\text{Na}$, 491.3496); ESIMS/MS m/z 423.9 $[\text{M}-\text{CO}_2\text{H}]^-$. *bis(TMS)-1*: EIMS m/z 612 $[\text{M}]^+$.

(–)-*Petrosynoic Acid B (2)*: clear oil; $[\alpha]_{\text{D}}^{24} -10.5$ (c 1.0, CHCl_3); ^1H NMR (600 MHz, methanol- d_4) and ^{13}C NMR (150 MHz, methanol- d_4) data, see Table 1 and Figure S5; ESIMS m/z 517.3658 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{33}\text{H}_{50}\text{O}_3\text{Na}$, 517.3652); ESIMS/MS m/z 449.7 $[\text{M}-\text{CO}_2\text{H}]^-$. *bis(TMS)-2*: EIMS m/z 638 $[\text{M}]^+$.

(–)-*Petrosynoic acid C (3)*: clear oil; $[\alpha]_{\text{D}}^{24} -12.5$ (c 1.0, CHCl_3); ^1H NMR (600 MHz, methanol- d_4) and ^{13}C NMR (150 MHz, methanol- d_4) data, see Table 1 and Figure S6; ESIMS m/z 515.3528 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{33}\text{H}_{48}\text{O}_3\text{Na}$, 515.3496); ESIMS/MS m/z 447.8 $[\text{M}-\text{CO}_2\text{H}]^-$. *bis(TMS)-3*: EIMS m/z 636 $[\text{M}]^+$.

(–)-*Petrosynoic acid D (4)*: clear oil; $[\alpha]_{\text{D}}^{24} -11.4$ (c 0.5, CHCl_3); ^1H NMR (600 MHz, methanol- d_4) and ^{13}C NMR (150 MHz, methanol- d_4) data, see Table 1 and Figure S7; ESIMS m/z 535.3744 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{33}\text{H}_{52}\text{O}_4\text{Na}$, 535.3759); ESIMS/MS m/z 467.8 $[\text{M}-\text{CO}_2\text{H}]^-$. *tris(TMS)-4*: EIMS m/z 728 $[\text{M}]^+$.

Pellynol A (5): spectroscopic data in accordance with published data.¹⁸

Pellynol C (6): spectroscopic data in accordance with published data.¹⁸

Pellynol D (7): spectroscopic data in accordance with published data.¹⁸

Pellynol F (8): spectroscopic data in accordance with published data.²²

Pellynol I (9): spectroscopic data in accordance with published data.²³

TMS Derivatization of Compounds 1–9

TMS derivatives of **1–9** were prepared by dissolving 0.1 mg of compound in 10 μ L dichloromethane, adding 20 μ L *bis*(trimethylsilyl)trifluoroacetamide (Aldrich) and letting stand at room temperature for 1 h. Products were analyzed directly by EIMS.

Cytotoxicity Assay

Cytotoxic effects of compounds **1–9** and vinblastine were evaluated in three human cancer cell lines as shown in Table 2. The cells were cultured in 96-well plates and grown in the absence or continuous presence of test compounds for 96 h. Cell growth was assessed using the CellTiter-Glo® luminescent cell viability assay (Promega). Luminescence was read on the EnVision 2102 MultiLabel Reader (Perkin-Elmer). IC₅₀ values were determined as the concentration of a compound at which cell growth was inhibited by 50% compared to untreated cell populations. Two separate replicate experiments were performed.

IMR-90 Cytotoxicity Assay

IMR-90 human fibroblasts, obtained from the American Type Culture Collection, were grown for 4 days to confluency in MEM containing 10% fetal bovine serum and supplemented with L-glutamine and penicillin/streptomycin. After washing, the medium was replaced with complete MEM containing 0.1% fetal bovine serum and cells were cultured for 3 additional days under these low serum conditions to achieve complete quiescence. Test compounds were then added followed by incubation for 96 h at 37°C. Cell viability was assessed by measurement of cellular ATP levels using CellTiter-Glo®.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Structures of (-)-petrosynoic acids A–D (1–4) isolated in this study.

Figure 2.
Molecular structures of known pellynols (**5–9**) isolated in this study.

compound	relative %	compound	relative %
petrosynoic acid A (1)	2.1	pellynol A (5)	33.3
petrosynoic acid B (2)	7.6	pellynol C (6)	9.9
petrosynoic acid C (3)	3.5	pellynol D (7)	5.1
petrosynoic acid D (4)	1.9	pellynol F (8)	2.4
		pellynol I (9)	34.2

Figure 3.

LC-ELSD trace for ASE extract XFM showing compounds **1–9** annotated with relative percentages of composition (Note: percent compositions derived from HPLC isolation yields). The LC was run at 1 mL/min flow rate with the following gradient: 50 – 100% CH₃CN in H₂O over 10 min, 100% CH₃CN for 10 min, 100 – 50% CH₃CN in H₂O over 2 min, and 50% CH₃CN in H₂O for 3 min (see Experimental for additional details).

Figure 4.

NMR traces of the XFM extract to highlight common terminal unit protons and acetylenic carbons, identical in melyne A and pellynol A (5), by showing ¹H peaks with key assignments and the ¹³C expanded region.

Figure 5. Substructural units A–F of compounds **1**, **2**, **3**, or **4** with selected COSY and HMBC correlations annotated.

Figure 6.

Diagnostic HREIMS fragments of (TMS)_n-petrosynoic acids **A–D (1–4)** with experimental *m/z* values annotated to justify the placement of substructures **C–F** within the acyclic frame.

Table 1

¹H (600 MHz) and ¹³C (150 MHz) NMR Data for Compounds 1 – 4 in methanol-*d*₄

1		2		3		4	
compd.							
pos #	δ _H (J [Hz])	δ _C	pos #	δ _H (J [Hz])	δ _C	pos #	δ _C
1	2.86, d (2.2)	74.5	1	2.86, d (2.2)	74.5	1	74.5
2		84.8	2		84.8	2	84.7
3	4.74, br d (6.2)	63.2	3	4.74, br d (6.3)	63.2	3	63.2
4	5.55, ddt (15.3, 6.3, 1.2)	130.6	4	5.55, ddt (15.2, 6.3, 1.2)	130.6	4	130.6
5	5.85, dtd (15.3, 6.7, 1.2)	134.2	5	5.85, dtd (15.2, 7.0, 1.2)	134.2	5	134.1
6	2.07, m	33.0	6	2.07, q (7.0)	33.0	6	33.1
7–13	1.25 – 1.53	29.0–31.0	7–13	1.25 – 1.53	29.0–31.0	7–15	29.0–31.0
14	2.04, m	28.1	14	2.12, t (6.6)	19.4	16	33.3
15	5.35, br t (4.9)	130.9	15		80.9	17	134.5
16	5.35, br t (4.9)	130.9	16		80.9	18	132.5
17	2.04, m	28.1	17	2.12, t (6.6)	19.4	19	73.8
18–25	1.25 – 1.53	29.0–31.0	18–27	1.25 – 1.53	29.0–31.0	20	38.5
26	2.38, q (7.44)	31.8	28	2.39, dq (7.5, 1.3)	31.7	21–27	29.0–31.0
27	6.27, dt (10.9, 7.6)	151.6	29	6.27, dt (10.9, 7.5)	151.4	28	31.5
28	5.61, br d (10.8)	107.7	30	5.61, br d (10.9)	107.8	29	147.6
29		86.9	31		NO ^a	30	109.0
30		82.9	32		82.7	31	91.9 ^b
31		157.5	33		157.6	32	77.3
						33	NO ^a

^aNO = not observed.

^bObserved in gHMBC.

Cytotoxic Activity and Relative Selectivity of Polyacetylenes 1–9 and Vinblastine Against Three Human Cancer Cell Lines and One Quiescent Human Fibroblast

Table 2

cell line:	A2058	H522-T1	H460	IMR-90	
cell type:	melanoma	lung	lung	fibroblast	SI _{A2058} ^b
compound	IC ₅₀ (μm)				
petrosynolic acid A (1)	3.7	5.5	3.4	0.4	0.1
petrosynolic acid B (2)	6.4	8.4	7.3	0.4	0.1
petrosynolic acid C (3)	3.8	4.6	3.1	0.3	0.1
petrosynolic acid D (4)	8.1	> 10	> 10	0.2	0.0
pellynol A (5)	0.4	0.8	0.4	0.4	1.0
pellynol C (6)	0.4	0.6	1.0	0.3	0.8
pellynol D (7)	0.8	1.1	0.7	0.5	0.6
pellynol F (8)	1.3	2.7	1.2	1.6	1.2
pellynol I (9)	0.8	1.2	0.8	NT ^a	-
vinblastine	0.004	0.009	0.002	>1	> 250

^aNT = not tested.

^bSI_{A2058} = IMR-90/A2058