Biogenetically Diverse, Bioactive Constituents of a Sponge, Order Verongida: Bromotyramines and Sesquiterpene–Shikimate Derived Metabolites†,[1]

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From a Verongid sponge, family Aplysinellidae and most likely a member of an undescribed genus, we have isolated respectable quantities of a bromotyramine derivative, moloka'iamine (1) and of puupehenone (5) related compounds. When broken apart, the animal emits hydrogen cyanide. The structures were determined spectrally and by comparison with published data. Puupehenone-related metabolites 2 and 4–8 exhibit a variety of biological activities (cyotoxicity, antiviral, antifungal, immunomodulatory) to varying degrees. 1, 6-Addition to the conjugated dienone system of puupehenone (5) enhances some of the activities.

Sponges of the order Verongida possess distinct morphological and biochemical characteristics. The animals, frequently light-colored while alive in the ocean, turn progressively darker when exposed to the atmosphere, and their secondary metabolites are bromotyrosine derivatives of widely varying complexity.[2]

We first collected a yellow sponge that gradually turned greenish-brown after it was removed from the ocean at the pier of Kaunakakai Harbor, Moloka'i, in April, 1990. Its color change suggested to us that it was a Verongid sponge. We have subsequently collected the same sponge in waters south of Maui and on several O'ahu beaches.[3] A striking physiological property set this animal apart from other Verongid sponges which we have studied: the sponge when broken into pieces emits a strong odor of hydrogen cyanide (see Experimental Part).[4] Equally startling was the chemical makeup of this sponge, which is the subject of this report. A sponge previously identified as *Psammaplysilla purpurea* from Hawaiian waters fits the description of this sponge.[5] *P. purpurea* has also been collected in the Red Sea and at Fiji, and its chemistry has been studied.[6,7] Previous chemical studies on *P. purpurea* never yielded any information on isoprenoid secondary metabolites. The sponge is most closely comparable to the genera *Psammaplysilla* and *Pseudoceratina* (Porifera, Demospongiae, Verongida, Aplysinellidae) and most likely is a member of an undescribed genus.

Extraction of 2.8 kg (wet) of sponge twice each with ethanol and then methylene chloride yielded crude extracts that showed activity against a KB cancer cell line. The extracts were combined, reduced in volume, and partitioned, first against hexane, then methylene chloride, and finally butanol. The combined butanol phases were subjected to silica flash chromatography and eluted with ethyl acetate, followed by methanol. Moloka'iamine (1)[8] (Chart I) eluted with methanol as a light-brown amorphous powder and was purified by repeated recrystallization from methanol. Both butanol and aqueous phases yielded moloka'iamine (1) in gram quantities (0.25% wet). Its structure is typical of metabolites of verongid sponges. This class of compounds has been reported in the literature in over 30 publications. Compound 1 is often represented as a substructure, but it had never been reported as an independent entity.[9] EIMS showed $M^+ + 2$ and $M^+ + 4$ signals, indicating a dibromo compound. This information coupled with a 2H aromatic singlet at 7.30 ppm in the ^1H NMR spectrum suggested a dibromophenol. Irradiation experiments established the connectivities of the aliphatic portions of the molecule. The compound is a salt, as evidenced by an additional proton in the positive ion EIMS and a characteristic broad IR band of an ammonium ion at 3000 cm^{-1}. Integration of the exchangeable protons in the ^1H NMR spectrum indicated the presence of 6 NH protons. The side-chain methylenes were easily assigned by the presence of the heteroatoms. Treatment of

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(2) Bergquist, P. R.; Wells, R. J. In *Marine Natural Products;* Scheuer, P. J., Ed. Academic Press: New York, 1983; Vol. V, pp 1–22.

(3) The sponge was collected from a pier in Kaunakakai Harbor, Moloka'i, in April 1990, and from the south shore of Oahu island, Hawaii, at a depth of 15 m, on Sept 26, 1992. The sponge formed a compressible thick encrustation, the surface of which is sparsely conulose. The texture of the sponge is slightly rubbery, but the sponge is easily torn due to the presence of sand and spicular debris. The sponge is found in association with fouling organisms such as polychaete worms and calcareous algae. The sponge is fleshy and has dense homogenous tissue, the fibers of which appear to consist of a very fine pithlike material and are frequently packed with debris. The sponge is comparable in morphology to the genera *Psammaplysilla* and *Pseudoceratina* (Porifera, Demospongiae, Verongida, Aplysinellidae). A voucher specimen has been deposited at the Harbor Branch Oceanographic Museum, Fort Pierce, FL (Catalogue No. 003: 00852).

(4) The presence of hydrogen cyanide has been confirmed by colorimetery and by GCMS. Test strips for the semiquantitative determination of cyanide are available from EM Science, Gibbstown, NJ.

(5) Devaney, D. M.; Eldredge, L. G. *Reef and Shore Fauna of Hawaii;* Bishop Museum Press: Honolulu, Hawaii, 1977; p 58.

(6) Rotem, M.; Carmely, S.; Kashman, Y. *Tetrahedron* **1983**, *39*, 667–676.

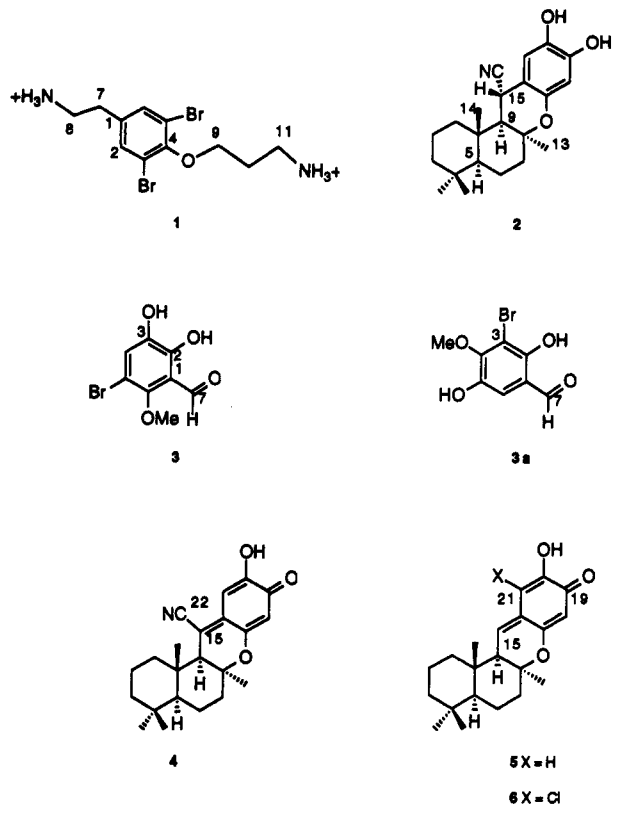
(7) Jimenez, C.; Crews, P. *Tetrahedron* **1991**, *47*, 2097–2102.

(8) This compound is named after the Hawaiian island of Moloka'i were the sponge was first collected, with help of the air compressor and the friendly members of the Kaunakakai Fire Department.

(9) Blunt, J. W.; Munro, M. H. B. A. Database of the Literature on Marine Natural Products, Department of Chemistry, University of Canterbury, Christchurch, New Zealand: 30 records found containing key words; Verongida, Tyrosine derived, and Bromo.

0022-3263/93/1958-6565$04.00/0

Chart I



moloka'iamine with 0.1 M methanolic silver nitrate solution yielded an off-white precipitate, which indicated that the anion was chloride.

The ethyl acetate eluates of the butanol partition resulted in the isolation of a slightly tan-colored glass (0.003%), cyanopuupehenol (**2**), which has been the subject of a preliminary communication.[10]

The combined hexane and methylene chloride partition fractions yielded a number of compounds by repeated preparative and semipreparative HPLC on RP-C18 columns. The most polar of six compounds (**3**–**8**) isolated from the lipophilic fractions (H_2O/MeOH, 85:15 for final purification) was 5-bromo-2,3-dihydroxy-6-methoxybenzaldehyde (**3**), in a yield of 0.0001%. The ^1H and ^{13}C data of **3** matched those of an alkaline degradation product (**3a**) of the psammaplysins.[6] On the basis of new NMR experiments not available in 1983, the structure of the benzaldehyde should be **3** rather than **3a**.[6,11] A coupling constant of 18 Hz between H7 and C1 distinguishes the two-bond HMBC correlation from the three-bond correlation. HMQC and HMBC experiments were used to assign the substitution pattern on the ring. HMBC data showed that the aromatic proton must be *para* to C1 which was established as being α to the aldehyde. A very sharp OH signal in the ^1H NMR spectrum at 11.73 ppm, characteristic of hydrogen bonded to the aldehydic oxygen, gave very distinct HMBC correlations. Bromo and methoxy positions were assigned by comparison with calculated ^{13}C chemical shift values.[12]

Next to be eluted with H_2O/MeOH (85:15) was cyanopuupehenone (**4**), isolated as a yellow-colored glass in very small yield (0.0001%). The EI mass spectrum of **4** provided a molecular formula of $C_{22}H_{27}NO_3$, comprising 10 elements of unsaturation as compared with 9 for cyanopuupehenol (**2**). The additional unsaturation suggested that **4** resulted from oxidation of cyanopuupehenol (**2**) to the corresponding quinone methide. IR (2205 cm^{-1}) and ^{13}C NMR (C15, 138.9 ppm, and C22, 115.6 ppm) data again provided evidence for the cyano function and for a single carbonyl (C-19, 181.6 ppm).

The major lipophilic component (0.11%) was isolated as a yellow glass and was identified as puupehenone (**5**).[13] ^1H and ^{13}C NMR spectra indicated a sesquiterpene–benzenoid system with four singlet methyls and a single carbonyl. A characteristic olefinic doublet (6.65 ppm) coupled to an aliphatic proton at 2.04 ppm (H-9) combined with its yellow color suggested that the **5** was puupehenone. Its optical rotation in CCl_4 was +297° as compared with a rotation of +315°, reported in the literature,[13] indicating identity. A total synthesis of puupehenone from sesamol and farnesyl bromide has been reported.[14] 21-Chloropuupehenone (**6**) was eluted (0.0001%) after elution of **5**. It was identified by comparison with reported spectral data.[15]

Since puupehenone was a major (0.11%) metabolite of this sponge, it occurred to us that **2** might have resulted from a 1,6 addition of HCN to **5**. We also noticed in the workup of puupehenone that it is possible to generate a methanol adduct, when puupehenone is cooled to dry ice temperature.[15] Warming the methanol adduct regenerates puupehenone quantitatively. Similarly, we have been able to generate **2** by treating puupehenone (**5**), dissolved in isopropyl alcohol, with HCN at 0 °C. The stereochemistry of the semisynthetic compound was identical with natural **2**, on the basis of comparing ^1H and ^{13}C NMR spectra. It is important to note that the coupling constant of H9 and H15 is nearly zero, thus indicating a dihedral angle of 90°. The coupling constant of the corresponding protons of the methanol adduct also approaches zero, thereby indicating that addition to the π system of puupehenone must be sterically favored from the opposite face of the terpenoid skeleton.

Puupehedione (**7**) was eluted on RP-C18 with H_2O/MeOH (9:1) as a deep red glass in a yield of 0.003%. A strong $M^+ + 2$ peak in the mass spectrum is a characteristic of *ortho* quinones regardless of the substitution pattern on the quinone ring.[16] Compound **7** shows this characteristic ion in the mass spectrum ($M^+ + 2$, 21), (M^+, 16). HMBC and NOE experiments established the structure and relative stereochemistry of this compound. The IR spectrum indicated that **7** no longer has an OH group. We suspect that this compound may be the result of the ready oxidation of this sponge. When this sponge is stored in EtOH next to another puupehenone-containing sponge, it is possible to observe the extracts turn from yellow, the color of puupehenone, to brick-red over a period of days.

A symmetrical dimer (**9**) of puupehenone has been reported in the literature.[15] We have isolated an asym-

(10) Hamann, M. T.; Scheuer, P. J. *Tetrahedron Lett.* **1991**, *32*, 5671–5672.

(11) Roll, D. M.; Chang, C. W. J.; Scheuer, P. J.; Gray, G. A.; Shoolery, J. N.; Matsumoto, G. K.; Van Duyne, G. D.; Clardy, J. *J. Am. Chem. Soc.* **1985**, *107*, 2916–2920.

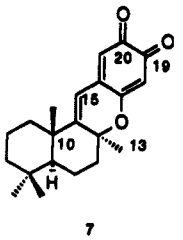
(12) Pretsch , E.; Clerc, T.; Seibl, J.; Simon, W. *Spectral Data for Structure Determination of Organic Compounds*, Springer-Verlag Press: Berlin, Heidelberg, 1983; pp C120–C125.

(13) Ravi, B. N.; Perzanowski, H. P.; Ross, R. A.; Erdman, T. R.; Scheuer, P. J.; Finer, J.; Clardy, J. *Pure Appl. Chem.* **1979**, *51*, 1893–1900.

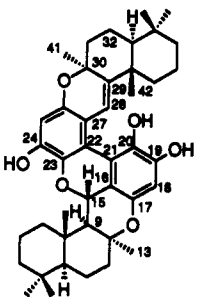
(14) Trammell, G. I. *Tetrahedron Lett.* **1978**, *18*, 1525–1528.

(15) Amade, P.; Chevelot, L.; Perzanowski, H. P.; Scheuer, P. J. *Helv. Chim. Acta.* **1983**, *66*, 1672–1675.

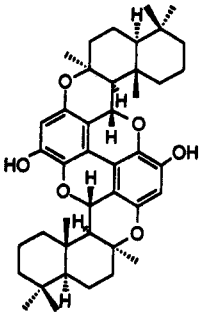
(16) Patai, S. *The Chemistry of the Quinoid Compounds*; Wiley and Sons: New York, 1974; p 238.



7



8



9

metric dimer, dipuupehetriol (8), eluted on RP-C18 with H_2O/MeOH (95:5) . The EI mass spectrum of this compound suggested a molecular formula of $C_{42}H_{54}O_6$ and hence the possibility that this compound was a puupehenone dimer. It moves very slowly on reverse phase chromatography, as one might predict, since there is an additional cyclic ether and its hydroxy groups are surrounded by alicyclic moieties. This compound was isolated as a slightly purple-colored solid in a yield of 0.001% and the structure, including relative stereochemistry, was secured by HMQC, HMBC, and ROESY data. The NMR spectra of 8 are much more complicated than those of 9 since dipuupehetriol is not symmetrical. The ^1H NMR spectrum of 8 shows eight different methyl groups and six signals from 4 to 7 ppm as opposed to 9 with only four methyl signals and two signals in the 4–7-ppm region. HMBC correlations of H15 to C16, C17, C21, and C23, in addition to a ROESY correlation between H28 and the OH at C20, were important for establishing the structure of this dimer. The bond between quaternary centers C21 and C22 was the last to be assigned. C22 is too far removed from any protons in the molecule to allow for their correlation by an HMBC experiment. There remained but one reasonable solution, to form a six-membered ring by joining C21 to C22. This satisfied the ^{13}C NMR chemical shifts (116.1, 119.6 ppm), results of the DEPT experiment, and degrees of unsaturation.

All eight compounds were tested for biological activity (Tables I and II). Both puupehenone and chloropuupehenone exhibit similar biological activities. In addition to exhibiting cytotoxicity at concentrations of <1 μg/mL in most of the cell lines tested, they also inhibit protein, RNA, and DNA synthesis at the same relative concentrations. These compounds also inhibit the activity of a number of enzymes including adenosine deaminase (ADA), glutathione reductase (GR), dihydrofolate reductase (DHFR), thymidylate synthetase (TS), and topoisomerase II (Topo II). Addition of HCN to puupehenone to generate cyanopuupehenol (2) has the effect of decreasing its cytotoxicity slightly, while enhancing its antiviral activity against HIV II. This reduction in cytotoxicity is still present, even when cyanopuupehenol is oxidized to cyanopuupehenone, which shows slight selectivity for human lung cancer (A549). Among all the compounds, puupehedione (7) shows the strongest immunomodulatory activity and no apparent antiviral activity. Bispuupehenone (9) was tested for cytotoxicity and showed no activity against any of the cell lines listed in Table I at a concentration of 20 μg/mL. In contrast, dipuupehetriol (8) shows distinct selectivity against cell lines A-549 and CV-1. These data suggest that additional 1,6 addition products of puupehenone should be investigated for structure–activity relationships.

One may speculate that the generation of HCN by the sponge might be a defensive measure and puupehenone may provide a mechanism for detoxification of HCN in the tissue of the sponge. As seen in Table I, the cyanocatechol system exhibits enhanced biological activity against HSV II virus and decreased cytotoxicity.

The chemistry of this sponge[3] is extraordinarily rich and unprecedented. Its only typical Verongid constituent is the bromotyramine fragment 1, which is present in massive amounts, and perhaps the somewhat enigmatic benzaldehyde 3. The remaining six metabolites belong to a sesquiterpene–shikimate biogenetic type first exemplified by avarol,[17] which was isolated from *Dysidea avara*, and has since found to be of widespread occurrence in members of the order Dictyoceratida, family Thorectidae. A report that avarol inhibits reproduction of the HIV virus[18] received wide attention and has heightened the interest in this class of compounds.

The presence of free hydrogen cyanide, when the sponge is broken after removal from the ocean, is an important observation. Discovery of the source of HCN may shed light on the unsolved question of the origin of the isocyanide and related functions in marine sponges.[19]

Experimental Section

Collection and Extraction. The first collection of 2.8 kg (wet) of the sponge, in April 1990, was extracted twice each with ethanol and then methylene chloride, yielding a crude extract that showed activity against a KB cancer cell line. The extracts were combined, concentrated, and partitioned, first against hexane, then methylene chloride, and finally butanol.

The volatile compounds from a second collection made in February 1991 (100 g) were trapped in a condenser at −105 °C. The condensed vapors were analyzed by GCMS (Alltech Heliflex AT-1 column, 15 m x 0.25 mm, film thickness 0.25 μm) at various temperatures from room temperature up to 100 °C. By comparison of retention times and *m/z* values of the vapor generated by the sponge with HCN, it was possible to show that the sponge liberated HCN. No additional volatile compounds could be documented.

Moloka'iamine (1) was isolated from the MeOH fraction of a silica gel flash column (butanol partition) as a light brown noncrystalline solid. Recrystallization from MeOH yielded 7 g of an off-white powder. Physical properties: ^{13}C NMR (75 MHz, DMSO) δ 150.8 (C-4), 137.1 (C-1), 133.1 (C-2, 6), 117.5 (C-3, 5), 70.4 (C-9), 39.6 (C-8), 36.4 (C-11), 31.3 (C-7), 27.6 (C-10); ^1H NMR (300 MHz, CD_3OD) δ 7.30 (s, 2 H-2, 6), 3.84 (t, J = 5.6 Hz, 2 H-9), 3.07 (t, J = 7.7 Hz, 2 H-11) 2.92 (t, J = 7.8 Hz, 2 H-8), 2.70 (t, J = 7.8 Hz, 2H-7), 1.97 (m, J = 6.7 Hz, 2H-10); IR neat (NaCl): 3000 (s, br), 1592 (m, br), 1473 (m), 1459 (m), 1385 (w), 1261 (m), 865 (m), 739 (m) cm^{-1}; EIMS, *m/z* (relative intensity) 354.9 (M^+ + H + 4, 1), 352.9 (M^+ + H + 2, 3), 350.9752 (M^+ + H, 2) (calcd for $C_{11}H_{17}{}^{79}Br_2N_2O$ 350.9708), 324.9 (57), 322.9 (100),

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(19) Scheuer, P. J. *Acc. Chem. Res.* **1992**, *25*, 433–439.

Table I. *In Vitro* Biological Activity of Compounds 1–4

	assay (MIC, μg/mL)			
	1	2	3	4
cytotoxicity, μg/mL				
KB	50 (+4)	5 (+2)		
LOVO	10 (+4)			
P-388 IC50	5	2	2.5	5
A-549 IC50	10	2	2.5	5
HT-29 IC50	5	2	5	1–2.5
CV-1 IC50	10	2	2.5	5
antiviral, μg/mL (% reduction)				
Mv 1 Lu/HSV II	10 (92)	5 (98)		
CV-1/HSV-1	>80	>8	>10	>80
BHK/VSV	>80	>8	2	>80
immunomodulator				
MLR (IM activity; IC50)	39	3		5
LCV (cytotoxicity; IC50)	44	42		10
potency (LCV/MLR) (>25)	CYT	15		2
antifungal, 6-mm disk	250 μg/disk all neg	50 μg/disk		
Aspergillus oryzae		–		
Penicillium notatum		10 mm partial		
Trichophyton mentagrophytes		9 mm		
Saccharomyces cerevisiae		7 mm		
Candida albicans		9 mm		

Table II. *In Vitro* Biological Activity of Compounds 5–8

	assay (MIC, μg/mL)			
	5	6	7	8
cytotoxicity, μg/mL				
KB	5 (+4)	5 (+4)	1 (+2)	>10
LOVO	1 (+4)	1 (+4)		10 (+3)
P-388 IC50	0.25	0.2	1	5
A-549 IC50	0.5	0.5	1–2	1
HT-29 IC50	0.5	0.5	1–2	10
CV-1 IC50	0.5	0.5	1	0.25
ADA IC50	>25	>25		
GR IC50	–	6		
DHFR IC50	5	5		
TS IC50	8	3		
PROT. IC50	0.4	0.3		
DNA IC50	0.3	1		
RNA IC50	0.4	>1		
Topo II IC50		1		
antiviral, μg/mL (% reduction)				
Mv 1 Lu/HSV II	>10	>10	>10	>10
CV-1/HSV-1	>4	>4	>80	>8
BHK/VSV	>4	>4	>80	>8
immunomodulator				
MLR (IM activity; IC50)			2	44
LCV (cytotoxicity; IC50)			>50	50
potency (LCV/MLR) (>25)			>20	CYT
antifungal, 6-mm disk	50 μg/disk	50 μg/disk	250 μg/disk	250 μg/disk all neg
Aspergillus oryzae	25 mm	14 mm	17 mm	
Penicillium notatum	30 mm	14 mm	17 mm	
Trichophyton mentagrophytes	10 mm	8 mm	13 mm	
Saccharomyces cerevisiae	12 mm	neg	15 mm	
Candida albicans	11 mm	7 mm	10 mm	

320.9 (61), 294.9 (18), 292.9 (16), 273.0 (88), 271.0 (91), 264.8 (30), 262.8 (16), 81.9 (66), 80.9 (27), 79.9 (68), 78.9 (28); UV (MeOH) λ_{max} 206 (28 000), 284 (600) nm.

5-Bromo-2,3-dihydroxy-6-methoxybenzaldehyde (3) was isolated from the EtOAc fraction of a silica gel flash column (hexane partition). Final purification was accomplished by HPLC using silica gel RP-18 (MeOH/H_2O (85:15)), yielding 3 mg. Physical properties: ^{13}C NMR (75 MHz, $CDCl_3$) δ 194.9 (C-7), 152.4 (C-4), 148.8 (C-2), 141.9 (C-1), 125.7 (C-6), 115.0 (C-3), 105.1 (C-5), 63.6 (C-8); ^1H NMR (300 MHz, $CDCl_3$) δ 11.73 (s, OH on 2), 10.20 (s, H-7), 7.33 (s, H-6), 5.60 (br s, OH on 1), 3.93 (s, 3H-8); IR neat (NaCl) 3319 (m, br), 2921 (m, br), 1633 (s), 1469 (s), 1446 (s), 1275 (s), 1195 (s), 1069 (m), 950 (m) cm^{-1}; EIMS m/z (fragment, relative intensity) 247.98 (M^+ + 2, 97), 245.9524 (M^+, 100) (calcd for $C_8H_7{}^{79}BrO_4$ 245.9528); UV (MeOH) λ_{max} 204 (12 245), 220 (9630), 276 (4930), 374 (1605) nm.

15-Cyanopuupehenone (4) was isolated from the CH_2Cl_2 fraction of a silica gel flash column (hexane and CH_2Cl_2 partitions). Final purification was accomplished by HPLC using silica gel RP-18 (MeOH/H_2O (85:15)), yielding 3 mg. Physical properties: $[\alpha]_D$ +168.0° (*c* 0.082, MeOH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 181.6 [C-19], 160.9 (C-17), 149.4 (C-20), 138.9 (C-15), 117.7 (C-16), 115.6 (C-22), 107.4 (C-21), 102.1 (C-18), 79.7 (C-8), 56.3 (C-9), 53.6 (C-5), 41.9 (C-10), 41.3 [C-1, 3, or 7], 40.3 [C-1, 3, or 7], 39.2 [C-1, 3, or 7], 33.7 (C-11), 33.4 (C-4), 27.6 (C-13), 21.8 (C-12), 18.0 (C-2, C-6), 14.8 (C-14); ^1H NMR (300 MHz, $CDCl_3$) δ 7.08 [s, OH], 6.67 (s, H-21), 5.89 (s, H-18), 2.20 [m, H-7], 2.19 [s, H-9], 1.94 (m, H-1), 1.63 (m, H-7), 1.60 (m, 2 H-6), 1.51 (m, 2 H-2), 1.44 (m, H-3), 1.30 (m, H-3), 1.25 (s, 3 H-13), 1.20 (m, H-1), 0.97 (m, H-5), 0.93 (s, 3 H-11), 0.85 (s, 6 H-12, 14); IR neat (NaCl) 3332 (s, br), 2929 (m, br), 2205 (m), 1626 (s), 1598 (s), 1426 (m), 1389 (s), 1191 (m), 1156 (m) cm^{-1}; EIMS *m/z* (fragment, relative

intensity) 354.2 ($M^+ + 1$, 5) 353.2003 (M^+, 19) (calcd for $C_{22}H_{27}NO_3$ 353.1991), 202.1 (100), 203.1 [26], 191.2 (7), 177.1 (14), 165.1 (6), 135.1 (27), 123.1 (11), 121.1 (18), 107.1 (17), 105.1 (15), 95.1 (36); UV (MeOH) λ_{max} 204 (10 650), 348 (13 305), 530 (540) nm.

Puupehenone (5) was isolated from the CH_2Cl_2 fraction of a silica gel flash column (hexane and CH_2Cl_2 partitions). Final purification was accomplished by preparative HPLC using silica gel RP-18 (MeOH/H_2O (85:15)), yielding 3 g. Physical properties: $[\alpha]_D$ +297.0° (*c* 0.44, CCl_4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 182.0 [C-19], 162.8 (C-17), 147.5 (C-20), 140.4 (C-15), 129.3 (C-16), 106.1 (C-21), 105.1 (C-18), 78.8 (C-8), 54.8 (C-9), 53.8 (C-5), 41.6 (C-10), 40.7 [C-1, 3, or 7], 40.0 [C-1, 3, or 7], 39.2 [C-1, 3, or 7], 33.7 (C-11), 33.3 (C-4), 28.0 (C-13), 21.9 (C-12), 18.4 (C-2 or C-6), 18.1 (C-2 or C-6), 15.0 (C-14); ^1H NMR (300 MHz, $CDCl_3$) δ 6.90 [s, OH], 6.65 (d, J = 6.9 Hz, H-15), 6.20 (s, H-21), 5.86 (s, H-18), 2.17 [dd, J = 11.4, 2.7 Hz, H-7], 2.04 [d, J = 6.9 Hz, H-9], 1.70 (m, H-1), 1.56 (m, H-7), 1.54 (m, 2 H-6), 1.51 (m, 2 H-2), 1.45 (m, H-3), 1.40 (m, H-3), 1.23 (s, 3 H-13),1.15 (m, H-1), 0.96 (m, H-5), 0.91 (s, 3 H-14), 0.84 (s, 3 H-11), 0.82 (s, 3 H-12).

Chloropuupehenone (6) was isolated from the CH_2Cl_2 fraction of a silica gel flash column (hexane and CH_2Cl_2 partitions). Final purification was accomplished by HPLC using silica gel RP-18 (MeOH/H_2O (85:15)), yielding 3 mg. Physical properties: ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.8 [C-19], 162.5 (C-17), 144.2 (C-20), 141.0 (C-15), 127.5 (C-16), 110.9 (C-21), 105.0 (C-18), 79.1 (C-8), 54.7 (C-9), 53.8 (C-5), 41.6 (C-10), 41.0 (C-1, 3, or 7), 40.1 (C-1, 3, or 7), 39.0 (C-1, 3, or 7), 33.7 (C-11), 33.4 (C-4), 27.9 (C-13), 21.9 (C-12), 18.4 (C-2, C-6), 18.1 (C-2, C-6), 15.1 (C-14); ^1H NMR (300 MHz, $CDCl_3$) δ 7.23 [s, OH], 7.13 (d, J = 7.2 Hz, H-15), 5.83 (s, H-18), 2.18 (m Hz, H-7), 2.17 (d, J = 7.2 Hz, H-9), 1.72 (m, H-1),1.57 (m, H-7), 1.54 (m, 2 H-6), 1.51 (m, 2 H-2), 1.45 (m, H-3), 1.42 (m, H-3), 1.23 (s, 3 H-13),1.19 (m, H-1), 0.98 (m, H-5), 0.92 (s, 3 H-14), 0.85 (s, 3 H-11), 0.81 (s, 3 H-12); 362.1642 (M^+, 19) (calcd for $C_{21}H_{27}ClO_3$ 362.1648).

Puupehedione (7) was isolated from the CH_2Cl_2 fraction of a silica gel flash column (hexane and CH_2Cl_2 partitions). Final purification was accomplished by HPLC using silica gel RP-18 (MeOH/H_2O (90:10)), yielding 85 mg. Physical properties: $[\alpha]_D$ +208° (*c* 0.087, MeOH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 180.9 (C-20 or C-19), 179.4 (C-20 or C-19), 169.5 (C-9), 164.6 (C-17), 138.3 (C-16), 122.0 (C-21), 115.2 (C-15), 109.0 (C-18), 81.8 (C-8), 43.2 (C-5), 41.5 (C-3), 40.8 (C-10), 38.4 (C-1), 33.8 (C-4), 32.6 (C-12), 30.8 (C-13), 29.4 (C-7), 25.0 (C-14), 21.0 (C-11), 18.6 (C-2), 16.6 (C-6); ^1H NMR (300 MHz, $CDCl_3$): δ 6.30 (s, H-15), 6.11 (s, H-21), 5.95 (s, H-18), 2.05 (m, H-1), 2.02 (m, 2 H-7), 1.88 (m, H-6), 1.69 (m, H-6), 1.57 (m, 2 H-2), 1.52 (s, 3 H-13), 1.46 (m, H-3), 1.45 (m, H-5), 1.29 (m, H-1), 1.23 (s, 3 H-14), 1.13 (m, H-3), 0.94 (s, 3 H-11), 0.88 (s, 3 H-12); IR neat (NaCl) 2950 (m, br), 1735 (w), 1674 (w), 1654 (s), 1642 (m), 1603 (s), 1561 (s), 1391 (s), 1229 (s), 1132 (m), 1061 (m) cm^{-1}; EIMS *m/z* (fragment, relative intensity) 328.2 ($M^+ + 2$, 21), 326.1890 (M^+, 16) (calcd for $C_{21}H_{26}O_3$ 326.1882), 314.2 (22), 313.2 (100), 298.2 (22), 283.1 (13), 202.0 (65), 177.0 (42), 176.0 (23), 174.0 (26), 162.0 (19), 161.0 (22), 160.0 (30), 137.1 (18), 91.0 (24), 69.0 (39.0); UV (MeOH) λ_{max} 204 (12 000), 250 (20 000), 356 (11 000), 436 (1665) nm.

Dipuupehetriol (8) was isolated from the CH_2Cl_2 fraction of a silica gel flash column (hexane and CH_2Cl_2 partitions). Final purification was accomplished by HPLC using silica gel RP-18 (MeOH/H_2O (95:5)), yielding 25 mg. Physical properties: $[\alpha]_D$ -18° (*c* 0.136, MeOH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.4 (C-29), 147.3 (C-26), 147.2 (C-17), 147.0 (C-20 or C-19), 145.8 (C-23 or C-24), 138.6 (C-24 or C-23), 133.4 (C-19 or C-20), 119.6 (C-22), 116.1 (C-21), 111.5 (C-27), 111.3 (C-28), 111.2 (C-16), 105.1 (C-18), 103.0 (C-25), 76.4 (C-30), 75.8 (C-8), 72.8 (C-15), 57.1 (C-9), 54.8 (C-5), 44.0 (C-33), 41.9 (C-35), 41.8 (C-3), 40.1 (C-7), 39.43 (C-1), 39.40 (C-37), 39.0 (C-38), 37.8 (C-10), 33.9 (C-34), 33.6 (C-11 or C-12), 33.3 (C-4), 32.6 (C-39 or C-40), 30.7 (C-31), 27.5 (C-13), 24.8 (C-42), 24.1 (C-41), 22.0 (C-12 or C-11), 21.1 (C-40 or C-39), 18.7 (C-36), 18.1 (C-2 and C-6), 17.2 (C-32), 14.0 (C-14); ^1H NMR (500 MHz, $CDCl_3$) δ 6.59 (s, H-18), 6.54 (s, H-25), 6.13 (s, H-28), 5.86 (s, exchangeable, H-19), 5.67 (s, exchangeable, H-20), 5.62 (s, exchangeable, H-24), 4.38 (s, H-15), 2.25 (m, H-7), 2.21 (m, H-31), 2.06 (m, H-31), 2.06 (m, H-37),1.93 (m, H-32), 1.86 (m, H-1), 1.81 (m, H-9), 1.76 (m, H-32), 1.67 (m, H-7), 1.67 (m, H-6), 1.64 (m, 2 H-36), 1.58 (m, H-6), 1.48 (s, 3 H-41), 1.45 (m, H-33), 1.45 (m, H-35), 1.40 (m, 2 H-2), 1.40 (m, H-3), 1.35 (m, H-37), 1.21 (s, 3 H-42), 1.20 (m, H-3), 1.13 (s, 3 H-13), 1.12 (m, H-1), 1.12 (m, H-35), 1.00 (m, H-5), 0.97 (s, 3 H-39), 0.94 (s, 3 H-11), 0.90 (s, 3 H-40), 0.85 (s, 3 H-12), 0.82 (s, 3 H-14); IR neat (NaCl) 3430 (s, br), 2946 (s, br), 1595 (s), 1431 (s), 1371 (s), 1301 (m), 1265 (s), 1239 (m), 1134 (m) cm^{-1}; EIMS *m/z* (fragment, relative intensity) 655.5 ($M^+ + 1$, 14) 654.381 (M^+, 31) (calcd for $C_{42}H_{54}O_6$ 654.392), 641.5 (15) 640.5 (46), 639.5 (100), 503.3 (20), 81.1 (11), 69.1 (17); UV (MeOH): λ_{max} 206 (24 400), 230 (18 580), 292 (13 070), 342 (7760), 518 (1010) nm.

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Supplementary Material Available: ^1H and ^{13}C NMR spectra of compounds 1–4 and 7–9 (14 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.