Chemistry of Puupehenone: 1,6-Conjugate Addition to Its Quinone−Methide System

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Received May 15, 1998

The marine natural product puupehenone (**1**), isolated in good yields from sponges of the genus *Hyrtios*, has been shown to undergo stereospecific 1,6-conjugate addition to its quinone−methide system. Several nucleophilic agents such as hydrogen cyanide, Grignard reagents, and nitroalkanes were studied, producing structurally diverse compounds. This lead optimization study was initiated due to the bioactivity of puupehenone and its natural analogues, which includes numerous previous reports of potential anticancer and antiinfective activity.

Marine natural products have proven to be a rich source of novel compounds with a variety of biological activities.[1] Puupehenone (**1**) and its derivatives isolated from sponges of the order Verongida and genus *Hyrtios* collected around the world have attracted significant attention by several research groups due to their cytotoxic, antimicrobial, and immunomodulatory activities.[2−14] Puupehenone (**1**) belongs to a family of secondary metabolites constructed from a drimane sesquiterpene and a C_6-shikimate moiety, first exemplified by the quinol−quinone pair of avarol and avarone.[15] Structurally, puupehenone differs from typical natural sesquiterpene quinones[16] by having a quinone−methide system that is certain to be responsible for its unique chemical as well as biological behavior.[17] Natural quinone−methides are present in both terrestrial plants and marine organisms and play an important role as intermediates in many biochemical processes, including oxidative phosphorylation, biogenesis of lignans and lignins in plants, sclerotization of insect cuticles, and melanization in animal cells.[17,18] The importance of quinone−methide systems as bioreductive alkylating agents is well recognized,[17] and attention has recently been focused on the interaction of this type of compound with DNA.[19] Puupehenone (**1**) represents an ideal natural model to study these interactions. Its quinone−methide system is combined with a nonplanar drimane−sesquiterpene moiety that can alter the binding profile with DNA as compared to simple quinone−methides.

Because alkylation is the most probable process in interaction of puupehenone with DNA, we decided to study the 1,6-conjugate addition reaction to puupehenone with several nucleophiles. We chose hydrogen cyanide, Grignard reagents, and nitroalkanes as the carbon nucleophiles, and methoxylation as an example of oxygen nucleophilic addition, to be followed later with 1,6-conjugate addition of sulfur and nitrogen reagents. Studies of hydrogen cyanide conjugate addition to puupehenone were dictated by the fact that a Verongid sponge containing puupehenone emits hydrogen cyanide as a probable chemical weapon against predators.[9]

The serendipitous and unprecedented discovery of the emission of hydrogen cyanide from a harvested sponge after it has been broken apart[9] offered a unique opportunity to shed light on the conditions that may lead to hydrogen cyanide emission and, ultimately, on the nature of its biogenetic precursor. The co-occurrence of puupehenone and its two cyano derivatives[7,9] in a Verongida sponge may suggest that the nucleophilic addition of hydrogen cyanide in vivo is the process responsible for detoxification of excess HCN used for defense purposes. The preliminary observation that the addition of hydrogen cyanide to a solution of puupehenone in 2-propanol produces stereospecifically 15α-cyanopuupehenol (**2**) suggested that biogenetically, cyanide was not a part of the drimane portion of the structure, but rather reacted with the intact ring system of puupehenone.[9] Our present and thorough investigation of the reaction of hydrogen cyanide and its precursors with puupehenone examines various aspects of the possible origin of cyanide ion and of the conditions (pH, temperature) of the process. The biomimetic study of cyanide addition established the conditions for the evaluation of other nucleophiles.

The potent cytotoxicity and antimicrobial activity of puupehenone suggests that it also may act as a chemical defense in the sponges that do not emit HCN, although there is no currently published study to support this. Our biomimetic experiments were designed to support the hypothesis that puupehenone may have originally been biosynthesized as a chemical defense and then later provided the mechanism for the evolution of a symbiotic relationship with a cyanide-emitting microorganism.

Results and Discussion

The reaction of cyano compounds with puupehenone was conducted primarily in methanol (see Table 1). Acetone, ethyl acetate, and diethyl ether were also used to evaluate the impact of the protic/aprotic properties of the solvent on the reactivity of the system. The previously noted ability of methyl alcohol to form a conjugate adduct with puupehenone[12] did not seem to interfere or prevent the reaction of puupehenone with cyanide nucleophiles. Under all experimental conditions for the addition of hydrogen cyanide to puupehenone, the presence of water in the reaction mixture was a crucial element for the observed generation of a catechol or quinone−methide product. (See Scheme 1.)

When dry HCN[20] and anhydrous MeOH were the reagents during 24 h at room temperature, 15α-cyanopuupehenol (**2**) was the sole product in quantitative yields. When HCN contained trace quantities of H_2O,[21] however,

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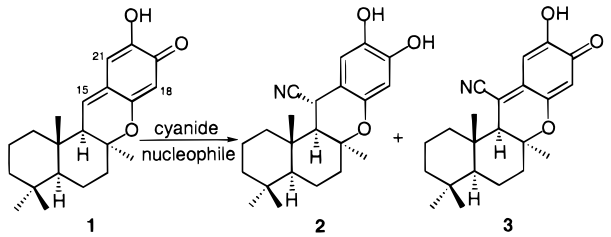
10.1021/np9802062 CCC: $15.00
Published on Web 10/31/1998

Table 1. 1,6-Conjugate Addition of Cyanides to Puupehenone

cyanide nucleophile	Mol. excess of cyanide	solvent	temperature (°C)	pH	time	product ratio **2**:**3**	yield (%)
HCN wet	> 100	acetone	r.t.[a]	5	30 h	1:0	20[b]
HCN wet	> 100	MeOH	0−5	4	24 h		no reaction
HCN wet	> 100	MeOH	r.t.	4	24 h	4:1	100
HCN anh.	> 100	MeOH	r.t.	4−5	24 h	1:0	100
HCN	> 100	aqueous Florisil	25−35 (gradient)	8.0[c]	17.5 h	1:1	96
NaCN	7	ether	r.t.	8	1 h		no reaction
NaCN	5.5	EtOAc	r.t.	8	3 h		no reaction
NaCN	5.5	acetone	r.t.	8	30 min	1:2	50[a]
NaCN	6	MeOH	r.t.	8−9	50 min	0:1	92
$(CH_3)_2C(OH)CN$	8.5	ether	r.t.	8	72 h		no reaction
$(CH_3)_2C(OH)CN$	8.5	MeOH	r.t.	10	30 min	1:2	81
$CH_3CH(OH)CN$	13	MeOH	r.t.	10	50 min	0:1	28
$HOCH_2CN$	19	MeOH	r.t.	10	50 min	0:1	11

[a] r.t. = room temperature. [b] Plus recovered unchanged starting material. [c] Monitored by pH meter.

Scheme 1



the reaction with puupehenone under the same conditions provided a mixture of 15α-cyanopuupehenol (**2**) and 15-cyanopuupehenone (**3**) in a ratio of 4:1. Lowering the reaction temperature resulted in slowing the reaction progress, and no detectable amounts of compounds **2** or **3** were observed after 24 h at 0−5 °C; starting material was fully recovered from the reaction mixture.

Similarly, substitution of MeOH with Me_2CO substantially slowed the reaction. Partial addition (up to 20% yield)[22] was observed when an Me_2CO solution of puupehenone saturated with wet HCN was left for 30 h at room temperature. Small (C_1−C_3) cyanohydrins were evaluated as a possible source of cyanide nucleophiles for the reaction with puupehenone. Hydroxyacetonitrile, 2-hydroxypropionitrile, and 2-hydroxyisobutyronitrile underwent rapid reaction with a methanolic solution of puupehenone at room temperature in the presence of 1N aqueous NaOH; within 30 to 50 min a dark-violet intermediate was formed. In all three reactions, partitioning of this colored intermediate between 5% aqueous $KHSO_4$ and ether afforded mostly 15-cyanopuupehenone (**3**). 2-Hydroxyisobutyronitrile was the most effective cyanide carrier, providing a solid intermediate that upon acidification yielded 81% of a mixture of 15α-cyanopuupehenol (**2**) and 15-cyanopuupehenone (**3**) in a 1:2 ratio.

The protic solvent MeOH participates significantly in the reaction. When MeOH was replaced by Et_2O, the reaction of 2-hydroxyisobutyronitrile with puupehenone and 1N aqueous NaOH in a two-phase system did not produce detectable amounts of product, even after prolonged stirring at room temperature. The reaction of puupehenone with sodium cyanide in an Me_2CO solution at room temperature for 30 min yielded the same dark-violet intermediate as the cyanohydrins. An acidic workup (5% $KHSO_4$, ether) provided 50% of a mixture of 15α-cyanopuupehenol (**2**) and 15-cyanopuupehenone (**3**) in 1:2 ratio, and 50% unchanged starting material **1**. When the reaction of sodium cyanide with puupehenone was carried out in MeOH, a colored intermediate was also formed, providing, after acidification with $KHSO_4$, 15-cyanopuupehenone (**3**) as the sole product of the reaction in nearly quantitative yield. Formation of the dark-violet intermediate was studied by carrying out the reaction in deuteriomethanol under anhydrous conditions and under positive nitrogen pressure using a 20% molar excess of higher quality (97%) sodium cyanide. The ^1H NMR spectrum of the dark-violet intermediate revealed two singlets at 6.53 and 6.09 ppm and a benzylic singlet proton at 3.92 ppm. This pattern clearly corresponds to a 15-cyanopuupehenol−catechol system. Contributing to about 20% of the total integration were two signals at 6.33 and 5.62 ppm, which were assumed to be generated from 15-cyanopuupehenone (**3**). The presence of colored intermediates in the base-catalyzed addition of cyanides to puupehenone can be attributed to the formation of the corresponding sodium salts. A similar phenomenon was observed by other authors in structurally related sesquiterpene/quinones (spongiaquinones).[23]

Reactivity of puupehenone toward the cyanide nucleophile was also evaluated in aqueous solutions, partially mimicking the pH and temperature of the sponge's natural environment. In this experiment Florisil (100−200 mesh) was used as the solid support for puupehenone in a two-phase reaction and as the medium for buffering the mixture at pH 8.0 (see Experimental Section). After addition of gaseous hydrogen cyanide, magnesium cyanide was expected to be formed and compete with dissociated hydrogen cyanide at pH 8 in aqueous solution. During the reaction, the pH of the mixture was monitored. There were no detectable changes of pH (±0.1), and equal amounts of 15α-cyanopuupehenol and 15-cyanopuupehenone were formed.

The presence of 15-cyanopuupehenone in all of the above-described experiments suggests the participation of the oxidation reaction in the overall process. Oxygen from the air or dissolved in water is the most probable oxidant in this process. The presence of water greatly facilitates the oxidation of 15α-cyanopuupehenol (see experiments with dry and wet HCN). The basicity of the reaction mixture significantly accelerates the oxidation of the catechol system.[24]

This would explain why the oxidation product (15-cyanopuupehenone) was predominant in the experiments with cyanohydrins under alkaline conditions and why it was the only product in the reaction of puupehenone with excess sodium cyanide (pH about 9). The formation of 15-cyanopuupehenone (**3**) during addition of sodium cyanide to puupehenone in deuterated MeOH may be due to the presence of small amounts of H_2O (up to 0.2%) in commercial CD_3OD.[25]

The unusual ease of oxidation of the 15α-cyanopuupehenol−catechol system under alkaline conditions was further supported by experiments in which 15α-cyano-

Table 2. Oxidation of 15α-Cyanopuupehenol (**2**) to 15-Cyanopuupehenone (**3**) at Room Temperature

solvent	Mol. excess of aqueous 1N NaOH	pH	time	yield of **3** (%)
ether	2.5	8	3 h	95
MeOH	2.5	10	50 min	98
MeOH	2.5	10	24 h	70
aqueous Florisil		9.6[a]	15 h	89

[a] Measured by pH meter.

puupehenol (**2**) was oxidized in 70% yield during 24 h, by contact with MeOH after addition of a small amount of 1N NaOH solution (see Table 2). Passing air through the reaction vessel further accelerated this oxidation. In this case the oxidation of 15α-cyanopuupehenol provided 15-cyanopuupehenone in 98% yield in 50 min. The same reaction in Et_2O produced 15-cyanopuupehenone in 95% yield in 3 h. 15α-Cyanopuupehenol was also easily oxidized (89% yield) in a two-phase reaction when supported on solid Florisil and suspended in H_2O at room temperature (pH 9.6).

Our experiments prove that the 1,6-conjugated nucleophilic addition of hydrogen cyanide to puupehenone (**1**) is the primary and dominating reaction pathway, regardless of the pH of the reaction mixture and the origin of cyanide nucleophile. The major product of this reaction, 15α-cyanopuupehenol (**2**), is accompanied by its oxidation product, 15-cyanopuupehenone (**3**). Water and alkaline conditions play an important role in facilitating the oxidation of 15α-cyanopuupehenol (**2**). The exceptional ease with which puupehenone accepts the cyanide nucleophile, especially under aqueous conditions, may help to determine the possible hydrogen cyanide−puupehenone cycle and highlight the biological function of puupehenone in the sponge's biochemical system.

The nucleophilic addition of Grignard reagents showed that puupehenone can be alkylated successfully with methylmagnesium or ethylmagnesium halides (see Table 3). Vinylmagnesium and ethynylmagnesium halides did not provide alkylation products; instead, significant decomposition of the reaction mixture was observed. Larger alkyl, cycloalkyl, allyl, phenyl, phenylalkyl, and phenylalkenyl magnesium halides reacted sluggishly, providing unstable, unidentified products.

Interestingly, the stereochemical course of the reaction with methylmagnesium and ethylmagnesium halides depends on an excess of Grignard reagent. The addition of 2.3 molar equivalent of 0.5 M ethereal solution of methylmagnesium iodide to puupehenone produced 15α-methylpuupehenol (**4**) as the only product, which was later converted to more stable diacetate **5**. However, when a higher concentration (3.0 M) and a larger excess (8.2 molar equivalent) of methylmagnesium iodide was used, we found only 15β-methylpuupehenol (**6**) in the crude reaction mixture. Reaction with ethylmagnesium bromide similarly produced corresponding 15α-ethyl **7** and **8** and 15β-ethyl **9** isomers. (See Scheme 2.) Both pairs of epimers **4** vs **6** and **7** vs **9** are easily distinguishable by their ^{1}H NMR spectra. With 15α-isomers, the benzylic proton H-15 resonates as a sharp quartet **4** or triplet **7**, while with 15β-isomers these signals are split again by H-9 and H-21. Second, for 15β-epimers the aromatic protons H-18 and H-21 are shifted upfield (ca. 0.5 ppm), with proton H-21 showing a doublet (J = 2 Hz) due to allylic coupling with proton H-15. Molecular modeling[26] clearly shows that H-15 in 15α-isomers forms a near 90° dihedral angle (calcd 93.4°) with proton H-9 (J = 0 Hz), while this angle in the 15β-isomers is about 45° (calcd 46.0°) (J = 3 Hz), with similar dihedral angles between H-15 and H-21. It is not clear why the larger excess of Grignard reagent changes the stereochemistry of the Michael-type conjugate alkylation, but it is possible that extensive complexation of all three oxygen atoms in the puupehenone molecule creates enough steric hindrance for alkylation of carbon 15 from the α-side. It is well-known that organomagnesium reagents are often present as clusters or aggregates, especially alkylmagnesium bromides or iodides show concentration-dependent behavior.[27] It is also noted that diastereoselective and enantioselective conjugate addition of organometallic compounds, including Grignard reagents, were observed due to their chelating properties.[28]

Conjugate additions to puupehenone require especially careful selection of bases used for the generation of nucleophilic reagents. Our first attempts to react puupehenone with nitroalkane anions generated in situ by sodium methoxide or sodium acetoxide were unsuccessful, producing only decomposition products. Stability studies of puupehenone in different bases have shown that as little as 1 h at room temperature with sodium or potassium hydroxide or even potassium carbonate in MeOH leads to significant decomposition of puupehenone. Magnesium, calcium, and barium hydroxides form relatively stable salts with puupehenone, and recovery of puupehenone is almost quantitative after acidification with sodium hydrogen sulfate solution. These observations led us to successfully utilize commercial magnesium methoxide as the base for in situ generation of nitroalkane nucleophiles in the reaction with puupehenone. Using these conditions we were able to react puupehenone with nitromethane and nitroethane to form corresponding products **10** and **12**, identified as their diacetates **11** and **13** (see Table 3 and Scheme 3).

Products of the conjugate addition of oxygen nucleophiles such as acetoxy and methoxy ions to puupehenone were reported earlier.[12,13,29] Although the product of acetylation of puupehenone (15α-acetoxypuupehenol diacetate) was easily reproducible, we were never able to obtain 15α-methoxypuupehenol by simple addition of methanol. Although puupehenone−methanol adducts are mentioned in the literature,[10,12,30] no experimental procedure or full physical or spectral properties of this compound were described, most probably because of its instability.[29] Surprisingly enough, Russian authors were able to obtain 15α-methoxypuupehenol by simple crystallization of puupehenone from aqueous methanol as an artifact during chromatographic separations of the crude extract from an unidentified sponge collected in the Indian Ocean near Mauritius.[30] Their product was further acetylated to provide 15α-methoxypuupehenol diacetate (**14**). We were able to obtain the same product **14**, accompanied by 15α-acetoxypuupehenol diacetate (**15**), from the reaction of puupehenone with magnesium methoxide in methanol, followed by acetylation of the crude products mixture with acetic anhydride−pyridine. (See Scheme 4.)

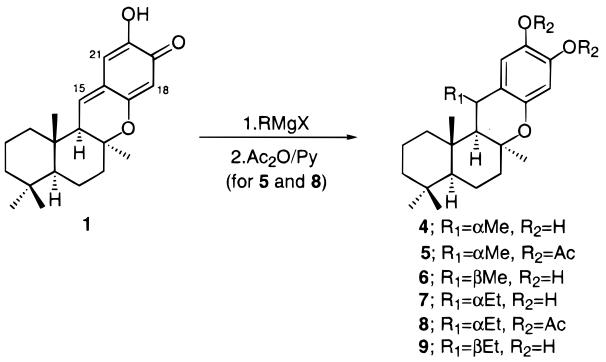
Puupehenone and its derivatives show a wide variety of biological properties, including cytotoxicity, antiviral, antifungal, antimalarial, and immunomodulatory activities.[6,7,9,11,13] Puupehenone was found to be active against such cancer cell lines such as P-388, A-549, HT-29, and melanoma at the concentration of $IC_{50} = 0.1-1.0$ μg/mL.[8,11] At similar concentrations puupehenone inhibits DNA and protein synthesis and also inhibits β-1,3-glucanase.[10] The derivatives produced by this study are currently being assayed both in vitro and in vivo for activity against HIV-1, AIDS−OI, other infectious diseases, and cancer. The

Table 3. 1,6-Conjugate Alkylation of Puupehenone with Grignard Reagents and Nitroalkanes

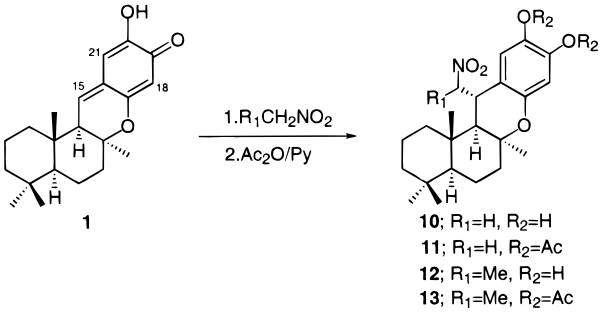
nucleophile	Mol. excess of Nucleophile	solvent	temp.	time	position (Nucleophile)	crude product no. yield (%)	acetylated product no. yield (%)
MeMgI	2.3	ether	0−r.t.[a]	1.5	α	**4**, 91	**5**, 43
MeMgI	8.2	ether	0−r.t.	1.5	*β*	**6**, 48	
EtMgBr	2.9	ether	0−r.t.	1.5	α	**7**, 67	**8**, 41
EtMgBr	8.2	ether	0−r.t.	1.5	*β*	**9**, 36	
CH_2═CHMgBr	8.2	ether	0−r.t.	1.5		dec.	
CH≡CMgBr	8.2	ether	0−r.t.	1.5		dec.	
CH_3NO_2[b]	4.0	benzene	0−r.t.	40	α	not isolated[c]	**11**, 72
$CH_3CH_2NO_2$[b]	4.0	benzene	0−r.t.	40	α	not isolated[c]	**13**, 32

[a] r.t. = room temperature. [b] Nucleophile generated by magnesium methoxide. [c] Unstable intermediate.

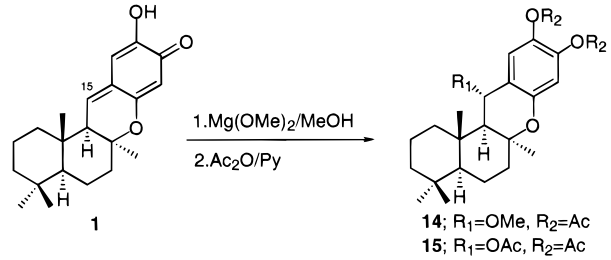
Scheme 2



Scheme 3



Scheme 4



results of the biological evaluation will be the subject of a separate report.

Experimental Section

General Experimental Procedures. Melting points (decompositions) were determined on a Thomas−Hoover capillary melting point apparatus and are uncorrected. NMR spectra were obtained on a Varian VXR-300 FT spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C NMR. Chemical shifts are reported in δ (parts per million) units relative to the internal reference tetramethylsilane (TMS). MS were obtained at the Mass Spectrometry Laboratory, University of Kansas, Lawrence, Kansas. EIMS data were obtained at 70 eV on a Nermag (Paris, France) R 10−10 quadrupole GC−MS spectrometer with SPECTRAL 30 data system. HRMSFAB were obtained at 8 keV on a ZAB HS mass spectrometer (VG Analytical Ltd., Manchester, UK) equipped with a 11/250 data system. Digital Corning pHmeter 320 with a Corning combination electrode cat. no. 476380 was used for monitoring pH. Analytical TLC was performed on Macherey & Nagel Alugram Sil G/UV 254 aluminum plates, developed in $CHCl_3$−EtOAc 10:1 v/v solvent system, and visualized with short-wave UV light (254 nm) or iodine vapors. Flash column chromatography was carried out on EM Science 230−400 mesh Si gel 60. The following adsorbents, solvents, and reagents were purchased from Aldrich Chemical Co.: Florisil 100−200 mesh, anhydrous Sure Seal methanol, methyl-d_3 alcohol-*d* (99.8% D), trifluoroacetic acid-*d* (99.5% D), potassium hydrogen sulfate (35−37% acidity as H_2SO_4), 2-hydroxyisobutyronitrile (99%, stabilized with H_2SO_4), 2-hydroxypropionitrile (98%, stabilized with 0.05% H_2SO_4), hydroxyacetonitrile (55 wt. % solution in H_2O, containing about 3 mol % CH_3OH). Certified (ACS) MeOH 99.9% and Me_2CO 99.6% were purchased from Fisher Scientific and used as solvents for the reactions without further purification; Et_2O with the peroxide content not exceeding 0.0001%, purchased from Fisher Scientific, was used as a solvent for the extractions; for reactions the same solvent was additionally distilled from metallic sodium. H_2O used for reactions and preparation of solutions was freshly distilled from a Corning AG-11 glass still. For the generation of gaseous HCN, purified, granular potassium cyanide from Fisher Scientific was used. Granular sodium cyanide 95% (Mallinckrodt) was used for the reactions in certified MeOH; however, for the reaction in deuterated MeOH, 97% NaCN (Aldrich) was applied.

Collection of Marine Organisms. The sponge *Hyrtios* sp. (2.5 kg) was collected by scuba from caves and piers (−30 ft) near the islands of O'ahu and Moloka'i, Hawaii.

Puupehenone (1). Sponge (2.5 kg) was freeze-dried for two weeks to afford semi-dried material (990 g). This material was extracted with MeOH (total amount 8 L) in a blender, the extracts were evaporated, and the wet residue was extracted with methylene chloride (4 L). Residual water (236 mL) was separated from the CH_2Cl_2 extracts, resulting in 754 g of dry sponge material present in the batch, 990 g of which represents 30% of the weight of the frozen animal. Methylene chloride extracts were evaporated, and the dry residue was re-extracted with warm (35 °C) petroleum ether (4 L) to afford 18.9 g of material with ca. 50% puupehenone (**1**) content (as evaluated by diagnostic peak integration in ^{1}H NMR). Florisil (200 g, 400 mL) was dry-packed on a chromatography column (52 × 425 mm) and flushed with starting eluent composition heptane−EtOAc (20:1) under 15 psi nitrogen pressure. Crude puupehenone (5.7 g, ca. 50% content) in $CHCl_3$ solution (12 mL) was loaded on the column and eluted with a gradient solvent mixture of heptane−EtOAc (up to 20:5) until UV transparent eluate was leaving the column (monitored by concentration of 100 mL of the eluate to the volume of 1 mL, spotted on Si gel TLC, and visualized in UV; total first eluate volume ca. 2.1 L). Further elution with the gradient solvent mixture heptane−EtOAc−HOAc (starting from 20:5:1 to 20:5:2) afforded puupehenone collected in 100-mL portions up to 500 mL as a yellow glass, 80−90 °C (dec), in the amount of 2.87 g. The residual 13.2 g of the crude material was purified

in the same way in three smaller portions giving, additionally, 6.54 g of the product, providing a total of 9.41 g (0.38% yield of the frozen and 1.2% of the dry sponge material) of puupehenone (**1**). To obtain an analytical NMR sample, a small portion of this product was rechromatographed on Si gel yielding puupehenone (**1**), mp 78−83 °C (dec); TLC (R_f = 0.58); HRFABMS *m/z* 329.2137 (MH^+ ; calcd for $C_{21}H_{29}O_3$, 329.2117). ^1H, ^{13}C NMR, and EIMS spectra were identical with literature data.[7,13]

15α-Cyanopuupehenol (2). Puupehenone (**1**) (166 mg, 0.5 mmol) was dissolved in anhydrous MeOH under positive pressure of dry nitrogen; the resulting solution was cooled to 0−5 °C in an ice-water bath, and anhydrous hydrogen cyanide gas[20] (approximately 0.1 mol as generated from solid KCN, 6.5 g, 0.1 mol and dried in $CaCl_2$ tube under positive stream of dry nitrogen) was passed through the solution for 5−6 min. The HCN generator was cut off, and the tightly closed reaction mixture was allowed to reach room temperature (25 °C) in 2.5 h and left at this temperature for additional 21 h. Evaporation and drying gave 180 mg (100%) of **2** as a green semisolid: mp 97−102 °C (dec); TLC (R_f = 0.33); ^1H NMR ($CDCl_3$) δ 6.77 (s, 1H, H-21), 6.34 (s, 1H, H-18), 3.85 (s, 1H, H-15), 2.08 (m, 1H), 1.80 (m, 1H), 1.72 (s, 1H, H-9), 1.63 (m, 1H), 1.59 (m, 2H), 1.50 (m, 2H), 1.44 (m, 1H), 1.33 (s, 3H, CH_3-13), 1.20 (m, 1H), 1.06 (m, 1H), 0.97 (m, 1H), 0.90 (s, 3H, CH_3-11), 0.80 (s, 3H, CH_3-12), 0.66 (s, 3H, CH_3-14); ^{13}C NMR δ 147.6, 145.4, 138.2, 122.3, 114.2, 106.3, 104.8, 74.9, 54.9, 54.4, 41.5, 40.3, 39.9, 38.8, 33.6, 33.2, 27.2, 24.3, 21.7, 18.2, 17.9, 13.7; EIMS *m/z* 355 $[M]^+$ (14), 341 (12), 338 (12), 326 (M − HCN, 21), 313 (92), 281 (62), 245 (17), 229 (21), 222 (25), 207 (25), 202 (17), 191 (8), 177 (54), 41 (100); HRFABMS *m/z* 355.2143 (M^+ ; calcd for $C_{22}H_{29}NO_3$, 355.2147).

15-Cyanopuupehenone (3). Puupehenone (**1**) (104 mg, 0.317 mmol) and sodium cyanide (97 mg, 2 mmol) in MeOH (0.6 mL) were stirred for 50 min at room temperature; the solvent was evaporated and the dark-violet intermediate was partitioned between Et_2O (5 mL) and 5% aqueous $KHSO_4$ (1 mL). The ethereal phase was evaporated to afford 104 mg (92%) of **3** as a light-brown semisolid: mp 57−62 °C (dec); TLC (R_f = 0.54); ^1H NMR ($CDCl_3$) δ 7.10 (br s, 1H, OH, exch. D_2O), 6.67 (s, 1H, H-21), 5.89 (s, 1H, H-18), 2.20 (m, 1H), 2.19 (s, 1H, H-9), 1.94 (m, 1H), 1.63 (m, 1H), 1.60 (m, 2H), 1.50 (m, 2H), 1.43 (m, 1H), 1.30 (m, 1H), 1.26 (s, 3H, CH_3-13), 1.21 (m, 1H), 1.00 (m, 1H), 0.93 (s, 3H, CH_3-11), 0.86 (s, 6H, CH_3-12 and CH_3-14); EIMS *m/z* 354 (MH^+, 48), 353 (M^+, 46), 338 (M - CH_3, 33), 284 (17), 271 (12), 243 (21), 242 (27), 220 (25), 215 (25), 202 (100); HRFABMS *m/z* 354.2050 (MH^+ ; calcd for $C_{22}H_{28}NO_3$, 354.2069).

Synthesis of 15-Cyanopuupehenone (3) in Deuterated Methanol, Monitoring the Reaction Mixture by ^1H NMR. The structure of the dark-violet intermediate was studied by carrying out the reaction in deuterated MeOH (CD_3OD) and monitoring by ^1H NMR. Puupehenone (**1**) (44 mg, 0.134 mmol) and sodium cyanide (8 mg, 0.163 mmol, 20% molar excess) were placed with a magnetic stirring bar in a 2-mL vial under a dry nitrogen. Deuterated MeOH (1 mL of CD_3OD) was added, and the reaction mixture was stirred for 19 h at room temperature. During that time the reaction mixture acquired a dark-violet color indicating the formation of an intermediate. Part of the reaction solution (0.7 mL) was transferred under dry nitrogen into an NMR tube via transferring needle: ^1H NMR (CD_3OD), (rel int vs **2**) δ 6.53 (s, 1H, H-21 in **2**), 6.33 (s, 0.25H, H-21 in **3**; exch. CD_3OD in 12 days), 6.08 (s, 1H, H-19 in **2**), 5.62 (s, 0.25H, H-19 in **3**), 3.92 (s, 1H, H-15 in **2**; exch. CD_3OD in 12 days), 2.14 (s, 0.25H, H-9 in **3**), 1.67 (s, 1H, H-9 in **2**), 1.30 (s, 3H, CH_3-13 in **2**), 1.25 (s, 0.75H, CH_3-13 in **3**), 0.94 (s, 0.75H, CH_3-11 in **3**), 0.92 (s, 3H, CH_3-11 in **2**), 0.87 (s, 1.5H, CH_3-12 and CH_3-14 in **3**), 0.83 (s, 3H, CH_3-12 in **2**), 0.70 (s, 3H, CH_3-14 in **2**).The residual reaction solution (0.1−0.2 mL, some loss noted in the transferring needle) was dissolved in deuterated MeOH (0.5 mL of CD_3OD), acidified by the addition of deuterated trifluoroacetic acid (0.2 mL), and transferred as above into an NMR tube: ^1H NMR (CD_3OD + CF_3CO_2D): δ 6.74 (s, 1H, H-21 in **2**), 6.67 (s, 1H, H-21 in **3**), 6.28 (s, 1H, H-19 in **2**), 5.77 (s, 1H, H-19 in **3**), 3.95 (s, 1H, H-15 in **2**), 2.23 (s, 1H, H-9 in **3**), 1.25 (s, 3H, CH_3-13 in **2**), 1.18 (s, 3H, CH_3-13 in **3**), 0.86 (s, 3H, CH_3-11 in **3**), 0.83 (s, 3H, CH_3-11 in **2**), 0.82 (s, 3H, CH_3-12 or CH_3-14 in **3**), 0.79 (s, 3H, CH_3-12 or CH_3-14 in **3**), 0.75 (s, 3H, CH_3-12 in **2**), 0.62 (s, 3H, CH3−14 in 2).

Reaction of Puupehenone (1) with 2-Hydroxypropionitrile. To a stirred solution of puupehenone **1** (43 mg, 0.130 mmol) and 2-hydroxypropionitrile (10 drops = 120 mg, 1.69 mmol) in MeOH (3 mL) 1 N aqueous NaOH (0.25 mL, 0.25 mmol) was added dropwise at room temperature. The dark-violet reaction mixture was stirred for 50 min and partitioned between Et_2O (10 mL) and 5% aqueous $KHSO_4$. The organic phase was separated, filtered through the short Si gel column, and evaporated to provide 13 mg (28%) of **3**.

Reaction of Puupehenone (1) with Hydroxyacetonitrile. In parallel with above conditions, puupehenone (**1**) (41 mg, 0.125 mmol) was treated with hydroxyacetonitrile (10 drops = 252 mg of 55 wt % aqueous solution, 138 mg of 100% hydroxyacetonitrile, 2.4 mmol) in MeOH (3 mL) for 50 min to yield 5 mg (11%) of **3**.

Reaction of Puupehenone (1) with 2-Hydroxyisobutyronitrile. Puupehenone (**1**) (40 mg, 0.12 mmol) and 2-hydroxyisobutyronitrile (8 drops, 90 mg, 1.05 mmol) were stirred in a two-phase system of Et_2O (5 mL) and 1 N aqueous NaOH (0.25 mL, 0.25 mmol) for 3.5 h at room temperature. No reaction was observed. Methanol (1 mL) was added, and stirring was continued for an additional 30 min. Evaporation of the reaction mixture gave a dark-violet intermediate, which was further partitioned between Et_2O (10 mL) and 5% aqueous KHSO4 (8 mL). The ethereal phase was separated and evaporated to afford 35 mg (81%) of a mixture of **2** and **3** in a 1:2 ratio.

Reaction of Puupehenone (1) with a Cyanide Nucleophile in Water. Puupehenone (**1**) (200 mg, 0.6 mmol) was dissolved in a heptane−EtOAc (5:1) solvent mixture (60 mL), and Florisil (8.0 g) was added in one portion. The suspension was stirred for 30 min at 35 °C; the solvents were evaporated, and the residue, puupehenone on Florisil (8.25 g), was dried in high vacuum for 30 min at room temperature. Distilled H_2O (100 mL) was added, and the suspension (pH 8.0) was stirred, cooled to 5 °C in an ice-water bath, and saturated with approximately 0.1 mole of hydrogen cyanide gas[21] for 5−7 min. When the HCN generator was cut off, the reaction mixture (pH 8) was allowed to warm to room temperature (25 °C) over 6 h and was further stirred for 14.5 h at the same temperature and additionally for 3 h at 35 °C. The suspension (pH 8.0, no change beyond 0.1 monitored by pH meter) was filtered, solids were dried in a vacuum for 30 min and extracted with a heptane−EtOAc−HOAc (40:10:2) solvent mixture (60 mL) to afford 206 mg (96%) of a mixture of **2** and **3** in 1:1 ratio.

Oxidation of 15α-Cyanopuupehenol (2) to 15-Cyanopuupehenone (3) in Methanol under Nitrogen Atmosphere. To a flask thoroughly flushed with dry nitrogen containing 15α-cyanopuupehenol (**2**) (26 mg, 0.075 mmol), MeOH (2.5 mL) was added in one portion followed immediately by an aqueous 1N NaOH solution (5 drops, 0.2 g, 0.2 mmol) via syringe at room temperature under a positive pressure of dry nitrogen. A slow change of color from light brown to light violet was observed within 12−15 h. After 24 h the reaction mixture acquired a dark-violet color; Et_2O (5 mL) and 5% $KHSO_4$ (5 mL) were added under nitrogen via syringe. The reaction mixture rapidly changed color to light yellow; an additional 5 mL of Et_2O was added, and the phases were separated. The ethereal phase was concentrated in vacuo, and the residue was purified on a Si gel column (9 × 35 mm) eluting with $CHCl_3$ to provide 18.5 mg (70%) of 15-cyanopuupehenone (**3**).

Air-Assisted Oxidation of 15α-Cyanopuupehenol (2) to 15-Cyanopuupehenone (3) in the Presence of NaOH in (a) Diethyl Ether, (b) Methanol and (c) Water. (a) To a solution of 15α-cyanopuupehenol (**2**), (53 mg, 0.16 mmol) in Et_2O (5 mL) stirred at room temperature (23 °C) in a broad-necked flask with a smooth flow of air inside, an aqueous 1N NaOH solution (0.4 *g* = 10 drops, 16 mg NaOH, 0.4 mmol) was added dropwise. The reaction mixture acquired a dark-

violet color within 3−5 min, with visible separation of a semisolid. Stirring at room temperature was continued for 3 h, maintaining the same level of Et_2O in the reaction flask (some loss noted during the flow of air). Aqueous 5% $KHSO_4$ (12.5 mL) was added, followed by Et_2O (5 mL), and stirring was continued until the ethereal phase acquired a light golden-brown color. The organic phase was separated; ether was removed, and the residue was dried to afford 50 mg (95%) of 15-cyanopuupehenone (**3**): TLC (R_f = 0.54).

(b) 15α-Cyanopuupehenol (**2**), (53 mg, 0.16 mmol) in MeOH solution (5 mL) was treated with aqueous 1N NaOH solution (0.4 *g* = 10 drops, 16 mg NaOH, 0.4 mmol) with access to air as described above, and the resulting stirred solution (23 °C, inside the flask, pH ca. 10 as measured by pHydrion 1−11 pH paper) changed color from brown to red and finally violet in 30 min. After an additional 20 min of stirring, Et_2O (5 mL) was added, followed by aqueous 5% $KHSO_4$ (12.5 mL), after which an immediate change of color from dark violet to light golden-brown was noted. The organic phase was separated; the aqueous phase was reextracted with Et_2O (5 mL), and the organic extracts were combined; the solvents were removed, and the residual yellow-brown glass was dried in a vacuum yielding 52 mg (98%) of 15-cyanopuupehenone (**3**).

(c) To a solution of 15α-cyanopuupehenol (**2**) in a solvent mixture of heptane (10 mL) and EtOAc (2 mL), Florisil 200 (2.0 g) was added in one portion, and the resulting suspension was stirred for 15 min in a water bath, at 40 °C. The solvents were removed in a vacuum; the residual mixture of 15α-cyanopuupehenol (**2**) on Florisil support was dried in high vacuum for 10 min, and distilled H_2O (60 mL, temperature 25 °C, pH 6.5 as measured by pH meter) was added in one portion with stirring in an open flask with an easy access to air. A smooth change to pH 9.6 was observed within 5−7 min, and also a color change of the mixture from brown to red-brown to violet was noted in 1.5 h. The reaction mixture was stirred for 15 h at room temperature (25 °C), acquiring a dark-violet color; Et_2O (20 mL) and aqueous 10% $KHSO_4$ (10 mL) were added with vigorous stirring, and a rapid change of color to light yellow was observed. The organic phase was separated, and the H_2O−Florisil suspension (pH ca. 2) was reextracted with Et_2O (2 × 10 mL). The organic extracts were combined; Et_2O was removed, and the residue was dried in high vacuum to afford 23 mg of 15-cyanopuupehenone (**3**). The solid phase (Florisil) was filtered from the H_2O suspension, dried in high vacuum, and washed with EtOAc (20 mL) on a small chromatography column. The eluate, after removal of EtOAc, contained an additional 24 mg of 15-cyanopuupehenone (**3**), providing a total of 47 mg (89%) of **3**.

15α-Methylpuupehenol (4) and Diacetate (5). To the cold (0 °C) solution of puupehenone (**1**) (25 mg, 0.076 mmol) in dry ether (1.5 mL) the 0.5 M ethereal solution of methylmagnesium iodide (0.36 mL, 0.18 mmol) was added slowly under nitrogen. The reaction mixture was stirred 1 h at 0 °C and for 30 minutes further at room temperature. After cooling to 0 °C, 5 mL of the wet ether was added to the reaction mixture followed by 5% aqueous $NaHSO_4$ until pH 7−8 was reached. The ether phase was then separated, washed with H_2O, dried over anhydrous sodium sulfate, and evaporated to dryness to afford 24 mg (91%) of the crude product **4**: TLC (R_f = 0.40); ^{1}H NMR ($CDCl_3$) δ 6.70 (s, 1H, H-21), 6.30 (s, 1H, H-18), 2.73 (q, *J* = 4.5 Hz, 1H, H-15), 1.36 (d, *J* = 4.5 Hz, 3H, CH_3-22), 1.15 (s, 3H, CH_3-13), 0.89 (s, 3H, CH_3-14), 0.80 (s, 3H, CH_3-11), 0.68 (s, 3H, CH_3-12).

The crude **4** was acetylated with Ac_2O−pyridine (1.8 mL, 2:1 v/v) to give, after column chromatography, 14 mg (43%) of 15α-methylpuupehenol diacetate (**5**): TLC (R_f = 0.66); ^{1}H NMR ($CDCl_3$) δ 6.98 (s, 1H, H-21), 6.56 (s, 1H, H-18), 2.82 (q, *J* = 7.5 Hz, 1H, H-15), 1.41 (d, *J* = 7.5 Hz, 3H, CH3−22), 2.25 (s, 3H, CH_3CO), 2.24 (s, 3H, CH_3CO), 1.17 (s, 3H, CH_3-13), 0.89 (s, 3H, CH_3-14), 0.81 (s, 3H, CH_3-11), 0.68 (s, 3H, CH_3-12); EIMS *m*/*z* 428 (M^+, 5), CIMS *m*/*z* 446 (M + H_2O, 47), 429 (MH^+, 3), 428 (M^+, 3), FABMS *m*/*z* 429 (MH^+, 50).

15β-Methylpuupehenol (6). To the cold (0 °C) solution of puupehenone (**1**) (24 mg, 0.073 mmol) in dry ether (1.5 mL) the 3.0 M ethereal solution of methylmagnesium iodide (0.2 mL, 0.6 mmol) was added under nitrogen. The reaction mixture was stirred 1 h at 0 °C and for 30 minutes further at room temperature. After cooling to 0 °C, 2 mL of the wet ether was added to the reaction mixture followed by 5% aqueous $NaHSO_4$, until pH 8 was reached. The reaction mixture was then extracted with ether (10 mL), the ether phase was separated, washed with H_2O, dried over anhydrous sodium sulfate, and evaporated to dryness to afford 49 mg of the crude product, which showed signs of decomposition. This crude product was further re-extracted with ether−petroleum ether (4 mL,1:1 v/v) and evaporated to provide 12 mg (48%) of unstable 15β-methylpuupehenol (**6**): TLC (R_f = 0.45); ^{1}H NMR ($CDCl_3$) δ 6.33 (d, *J* = 2.5 Hz, 1H, H-21), 5.80 (s, 1H, H-18), 2.73 (q, *J* = 4.5 Hz, 1H, H-15), 1.36 (d, *J* = 4.5 Hz, 3H, CH_3-22), 1.15 (s, 3H, CH_3-13), 0.89 (s, 3H, CH_3-14), 0.80 (s, 3H, CH_3-11), 0.68 (s, 3H, CH_3-12).

15α-Ethylpuupehenol (7) and Diacetate (8). Puupehenone (**1**) (34 mg, 0.103 mmol) was treated with 0.5 M ethereal solution of ethylmagnesium bromide (0.6 mL, 0.3 mmol) in the same manner as described for 15α-methylpuupehenol (**4**). After workup, 25 mg (67%) of the crude product **7** was obtained: TLC (R_f = 0.45); ^{1}H NMR ($CDCl_3$) δ 6.70 (s, 1H, H-21), 6.29 (s, 1H, H-18), 2.43 (m, 1H, H-15), 1.55 (m, 3H, CH_3-22), 1.15 (s, 3H, CH_3-13), 1.03 (t, 3H, *J* = 4.4 Hz, CH_3-23), 0.89 (s, 3H, CH_3-14), 0.80 (s, 3H, CH_3-11), 0.67 (s, 3H, CH_3-12).

The crude **7** was acetylated with Ac_2O−pyridine (1.8 mL, 2:1 v/v) to give after column chromatography 19 mg (41%) of 15α-ethylpuupehenol diacetate (**8**): TLC (R_f = 0.68); ^{1}H NMR ($CDCl_3$) δ 6.98 (s, 1H, H-21), 6.55 (s, 1H, H-18), 2.53 (m,1H, H-15), 2.24 (s, 6H, 2 × CH_3CO), 1.53 (m, 3H, CH_3-22), 1.17 (s, 3H, CH_3-13), 1.05 (t, 3H, *J* = 4.6 Hz, CH_3-23), 0.89 (s, 3H, CH_3-14), 0.81 (s, 3H, CH_3-11), 0.66 (s, 3H, CH_3-12); EIMS *m*/*z* 442 (M^+, 20), CIMS *m*/*z* 460 (M + H_2O, 75), 443 (MH^+, 8), 442 (M^+, 10), FABMS *m*/*z* 443 (MH^+, 37).

15β-Ethylpuupehenol (9). Ethereal solution (1.5 mL) of puupehenone (**1**) (24 mg, 0.073 mmol) was treated with the 3.0 M ethereal solution of ethylmagnesium bromide (0.2 mL, 0.6 mmol) in the same conditions as described for 15β-methylpuupehenol (**6**). After workup, 10 mg (36%) of unstable 15β-ethylpuupehenol (**9**) was obtained: TLC (R_f = 0.48); ^{1}H NMR ($CDCl_3$) δ 6.55 s,1H, H-21), 5.82 (s, 1H, H-18), 2.90 (m, 1H, H-15), 1.50 (m, 3H, CH_3-22), 1.25 (s, 3H, CH_3-13), 1.22 (t, 3H, *J* = 4.4 Hz, CH_3-23), 0.92 (s, 3H, CH_3-14), 0.87 (s, 3H, CH_3-11), 0.85 (s, 3H, CH_3-12).

15α-Nitromethylpuupehenol Diacetate (11). A solution (7.4%, 0.45 mL) of magnesium methoxide in MeOH [27.3 mg, 0.316 mmol of $Mg(OCH_3)_2$] was added to anhydrous C_6H_6 (15 mL), additionally pre-purged with dry nitrogen (15 min), followed by nitromethane (1 mL). This mixture was stirred for 1 h at room temperature, cooled to ice-water (0−5 °C) temperature, and a solution of puupehenone (104 mg, 0.317 mmol) in anhydrous nitrogen-purged C_6H_6 was added. The reaction mixture was stirred in an ice-water bath and allowed to reach room temperature within 18 h. After stirring for the next 6 h at room temperature, additional portions of magnesium methoxide in MeOH were added, and the reaction mixture was stirred until reaction was complete [total 0.40 mL of $Mg(OCH_3)_2$ in MeOH were used]. It is important that pH of the reaction mixture does not drop below 8 during an addition of magnesium methoxide solution. The reaction mixture was then stirred for the next 16 h at room temperature, cooled in an ice-water bath, and Ac_2O−pyridine mixture (3.7 mL, 2:1 v/v) was added. After stirring for 2 more hours at room temperature, the solvents were removed under vacuum, and the residue was purified on a Si gel column (toluene−$CHCl_3$ 7:3, v/v) to afford 108 mg (72%) of 15α-nitromethylpuupenenol diacetate (**11**) as semisolid material: TLC (R_f = 0.53); ^{1}H NMR ($CDCl_3$) δ 7.00 (s, 1H, H-21), 6.64 (s, 1H, H-18), 4.74 (m, 2H, CH_2NO_2), 3.58 (m, 1H, H-15), 2.25 (s, 6H, 2 × CH_3CO), 1.23 (s, 3H, CH_3-13), 0.88 (s, 3H, CH_3-14), 0.79 (s, 3H, CH_3-11), 0.67 (s, 3H, CH_3-12); EIMS *m*/*z* 473 (M^+, 4); FABMS *m*/*z* 474 (MH^+, 55), 473 (M^+, 60).

15α-Nitroethylpuupehenol Diacetate (13). Puupehenone (**1**) (104 mg, 0.317 mmol) was reacted with nitroethane

(1 mL) in a similar way as with nitromethane to afford 50 mg (32%) of 15α-nitroethylpuupenenol diacetate (**13**) as a semisolid material: TLC (R_f = 0.55); ^{1}H NMR ($CDCl_3$) δ 6.60 (s, 1H, H-21), 6.58 (s, 1H, H-18), 4.96 (m, 1H, $CH_3-CH-NO_2$), 3.38 (d, 1H, J = 10 Hz, H-15), 2.18 (s, 3H, CH_3CO), 2.17 (s, 3H, CH_3CO), 1.60 (d, 3H, J = 4.1 Hz, $CH_3-CH-NO_2$), 1.22 (s, 3H, CH_3-13), 0.87 (s, 3H, CH_3-14), 0.78 (s, 3H, CH_3-11), 0.62 (s, 3H, CH_3-12); EIMS m/z 487 (M^+, 10); CIMS m/z 505 ($M + H_2O$, 20); FABMS m/z 488 (MH^+, 58), 487 (M^+, 85).

15α-Methoxypuupenenol Diacetate (14) and 15α-Acetoxypuupehenol Diacetate (15). A solution of puupehenone (**1**) (100 mg, 0.3 mmol) in anhydrous ether (3 mL) was added under nitrogen to anhydrous C_6H_6 (10 mL) pre-purged with dry nitrogen. After cooling the mixture to 0 °C, the solution (7.4%, 0.3 mL, d = 0.816 mg/mL) of magnesium methoxide in MeOH [18 mg, 0.2 mmol of $Mg(OCH_3)_2$] was added dropwise. The reaction mixture was stirred at room temperature for 3 h, recooled to 0 °C, followed by addition of Ac_2O−pyridine (3.5 mL, 4:1 v/v). After stirring for another 15 h at room temperature, the reaction mixture was evaporated to dryness and the oily residue chromatographed on Si gel column. Elution of column with toluene gave 19 mg (13%) of 15α-acetoxypuupehenol diacetate (**15**): mp 146−148 °C, TLC (R_f = 0.65); ^{1}H NMR ($CDCl_3$) δ 7.06 (s, 1H, H-21), 6.65 (s, 1H, H-18), 6.01 (s, 1H, H-15), 2.25 (s, 3H, CH_3CO), 2.24 (s, 3H, CH_3CO), 2.05 (s, 3H, CH_3CO), 1.28 (s, 3H, CH_3-13), 0.88 (s, 3H, CH_3-14), 0.80 (s, 3H, CH_3-11), 0.67 (s, 3H, CH_3-12); EIMS m/z 472 (M^+, 7), FABMS m/z 473 (MH^+, 20), 472 (M^+, 60).

Identity of 15α-acetoxypuupehenol diacetate (**15**) was confirmed by independent acetylation of puupehenone yielding the product with mp 144−149 °C and NMR spectra in full accordance with published data.[13]

Further toluene eluates afforded 22 mg (16%) of 15α-methoxypuupehenol diacetate (**14**): TLC (R_f = 0.55); ^{1}H NMR ($CDCl_3$) δ 7.13 (s, 1H, H-21), 6.61 (s, 1H, H-18), 4.1 (s, 1H, H-15), 3.44 (s, 3H, CH_3O), 2.24 (s, 6H, 2 × CH_3CO), 1.21 (s, 3H, CH_3-13), 0.91 (s, 3H, CH_3-14), 0.81 (s, 3H, CH_3-11), 0.63 (s, 3H, CH_3-12).

Acknowledgment. We thank Dr. Paul Scheuer for editorial guidance, Dr. Todd Williams of the University of Kansas for mass spectra, Frank Wiggers for NMR spectral analyses, Keena Wesson and Tony Perry for their help with collections, and the Elsa U. Pardee Foundation, the Mississippi−Alabama Sea Grant College Program, the National Institute of Allergy and Infectious Diseases, and G.D. Searle for financial support.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1**−**15** as well as EIMS and HRMS−FAB spectra (30 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.

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NP9802062