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RECENT RESEARCH IN MARINE NATURAL PRODUCTS: THE PUUPEHENONES

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Abstract -- Isolation and structural elucidation of three sponge metabolites, puupehenone (4) and its monochloro (11) and monobromo (12) derivatives is described. The compounds are products of mixed biosynthesis and consist of a C_{15} unrearranged drimane and a 1,2,4-trihydroxybenzene moiety. Halogen is incorporated in the aromatic part of the molecule and the chlorine isomer predominates over the bromine compound. The structures are based on spectral data and on single crystal x-ray diffraction of a C_{18} ozonolysis product (1) of puupehenone (4). Puupehenone (4) possesses moderate activity against gram-positive bacteria and against fungi.

INTRODUCTION

Within the past few years sponges have become favorite research targets of marine natural products research (2,3). There are valid reasons for this distinction: the phylum Porifera, comprising the most primitive multicellular invertebrate animals, with an estimated 5000 described species, most of them marine, has circumglobal distribution; the animals are sessile and therefore easily collected; and the notion, supported by scattered data -- principally Bergmann's sterol research (4) -- that primitive organisms surpass complex ones in synthetic virtuosity. Although research into the metabolites of marine sponges has not so far yielded practical returns, it has confirmed our earlier surmise of the synthetic versatility of sponges and has unveiled a fascinating spectrum of new molecular structures, often rich in functionality (2,3).

In the course of our program of screening sponges for antibiotic activity we isolated a reasonably active compound of novel structure from a yellow encrusting sponge (5) which was first collected off the island of Lanai and subsequently in caves and on the underside of ledges at a depth of 10-15 m near the Blowhole, island of Oahu. We later encountered the same compound in a sponge collected at Enewetak Atoll in the Marshall islands, but in that case it was accompanied by two halogenated derivatives. Structural elucidation and reactions of these three compounds are the subject of this report (6). These sponges, in addition, elaborate well-characterized red, yellow, and colorless dimers, which are under investigation in our laboratory.

PUUPEHENONE

Methanol extraction of the fresh or frozen animal, followed by ether extraction of the aqueous concentrate yielded after preparative tlc (silica, chloroform) a lustrous yellow glass in about 0.5% yield of dry sponge. We named this compound puupehenone in honor of the beautiful princess Puupehe, whose legendary tomb (7) is located on top of a huge sheer lava rock off the leeward coast of Lanai where the sponge was first collected. An amorphous red pigment, $C_{42}H_{52}O_6$, was also isolated during preparative tlc. The Enewetak sponge yielded crystalline puupehenone, mp 129-130°, after work-up that included Florisil, Bio-Sil and Sephadex chromatography.

The antimicrobial activity of the pure compound is summarized in Table 1. Puupehenone is active against gram-positive bacteria and against *Candida*, *Trichophyton*, and *Trichomonas* species (8).

TABLE 1. In Vitro Activity of Puupehenone

Organism	M.I.C. in μg/ml
Staphylococcus aureus	1.2
Streptococcus pyrogenes	1.8
Escherichia coli	>50
Pseudomonas aeruginosa	>100
Candida albicans	3.1
Trichophyton mentagrophytes	1.6
Trichomonas vaginalis	3.1

Characterization of puupehenone, $C_{21}H_{28}O_3$, summarized below, pointed to a tetracyclic molecule possessing one carbonyl and three carbon-carbon double bonds and belonging to a group of compounds of mixed biogenetic (sesquiterpene + C_6) origin, since ^1H and ^{13}C nmr spectra bore no evidence of extraskeletal carbons.

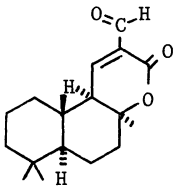
Rotation -- $[\alpha]_D$ + 315° (c 1.64, CCl_4).
Ir (CCl_4) -- 3380, 1630, 1615 cm^{-1}.
Uv (cyclohexane) -- 308 sh (21,600), 317 (24,300), 328 sh (16,000) nm.
^1H nmr ($CDCl_3$) -- δ 6.91 (bs, exchangeable in D_2O), 6.64 (1H, dd, J = 7, 1.5 Hz), 6.20 (1H, s), 5.86 (1H, d, J = 1.5 Hz), 2.03 (1H, d, J = 7 Hz), 3H singlets at 1.23, 0.92, 0.85, 0.83 ppm.
^{13}C nmr -- δ 181.8 s, 162.5 s, 147.2 s, 140.2 d, 129.0 s, 105.9 d, 105.0 d, 78.6 s, 54.7 d, 53.6 d, 41.5 t, 40.6 s, 39.8 s, 39.1 t, 33.6 q, 33.2 t, 27.9 q, 21.8 q, 18.1 t, 18.1 t, 15.0 q.
Ms (major fragments) -- 328 $C_{21}H_{28}O_3$; 313 $C_{20}H_{25}O_3$; 246 $C_{15}H_{18}O_3$; 231 $C_{14}H_{15}O_3$; 217 $C_{13}H_{13}O_3$; 204 $C_{12}H_{12}O_3$; 177 $C_{10}H_9O_3$; 148 $C_9H_8O_2$

Relevant chemical data included positive tests for enols (ferric chloride) and for reducing groups (silver oxide/silver nitrate or silver nitrate and periodate in nitric acid) (9). This information, together with spectral evidence, suggested two choices for the disposition of the three oxygen atoms in puupehenone: an enolized β-diketone plus an epoxide or an enolized α-diketone plus an enol ether of unspecified ring size or perhaps acyclic. A look at the major mass spectral fragments reveals that entry into the saturated hydrocarbon moiety of the molecule might prove difficult as the seven sp^2 carbons and the three hetero atoms of puupehenone almost certainly form a contiguous unit.

Since crystalline puupehenone from the Enewetak sponge did not become available to us until late in our investigation, we carried out a number of transformations that we hoped might lead to a crystalline derivative and thus define the unfunctionalized portion of the molecule. Ozonolysis at 0° with reductive work-up did indeed furnish a major product that was crystallized from heptane, mp 160-161°, and had composition $C_{18}H_{26}O_3$ as compared with $C_{21}H_{28}O_3$ for puupehenone. The C_{18} compound was spectrally characterized as an α,β-unsaturated lactonic aldehyde.

IR ($CHCl_3$) -- 1740 sh, 1710, 1700 cm^{-1}.
Uv (cyclohexane) -- 238 (10,500), 244 (10,500) nm.
^1H nmr ($CDCl_3$) -- δ 10.05 (1H, s), 7.68 (1Hd, J = 6.5 Hz), 2.19 (1H, d, J = 6.5 Hz), 1.24, 0.90, 0.84, 0.83 (3H s each) ppm.
^{13}C nmr -- δ 187.3 (d), 153.1 (d), 130.4 (s), 81.0 (s), 55.1 (d), 54.0 (d), 41.3, 40.7, 40.2, 39.0, 33.6, 33.4, 29.2, 21.8, 18.0, 18.0, 15.4 ppm. (No lactone carbonyl signal was recorded.)
Ms -- 290 $C_{18}H_{26}O_3$; 272 $C_{18}H_{24}O_2$; 257 $C_{17}H_{21}O_2$; 136 $C_8H_8O_2$, $C_{10}H_{16}$ (2 peaks).

The ozonolysis product, based on these and on X-ray crystallographic data, was assigned structure 1.



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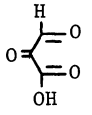
Preliminary X-ray photographs revealed that the ozonolysis product of puupehenone crystallized in the orthorhombic crystal class. Cell constants, determined by a least-squares fitting of fifteen reflections of moderate 2θ value, were $\underset{\sim}{a}$ = 7.813(2), $\underset{\sim}{b}$ = 13.728(3), $\underset{\sim}{c}$ = 15.103(2) Å. Systematic extinctions and the known chirality required space group $P2_12_12_1$. A calculated (z-4) and observed (flotation in aqueous ZnI_2) density of ∿1.2g/cc indicated one molecule of composition $C_{18}H_{26}O_3$ formed the asymmetric unit. All unique diffraction maxima (.92 Å resolution) were surveyed with a fully automated four circle diffractometer using a variable speed ω-scan and graphite monochromated CuKα (1.5418 Å) radiation. Of the 1302 reflections surveyed, 1129 (87%) were judged observed ($I \geq 3\sigma(I)$) after correction for Lorentz, polarization and background effects. The angular dependence of the scattering was removed and the data were reduced to normalized structure factors (10). Phases were assigned using a multisolution, weighted tangent formula approach and a plausible molecular fragment was found on one of the resulting E-syntheses. This fragment was extended to the complete nonhydrogen structure using the recycling procedure of Karle (11). Nonhydrogen atoms were assigned anisotropic thermal parameters and refined by full matrix least squares techniques. Most of the hydrogens were located on subsequent difference syntheses but some were included at calculated positions. Full matrix least squares refinements with anisotropic heavy atoms and isotropic hydrogens have converged to the current unweighted crystallographic residual of 0.039 for the observed reflections. Figure 1 is a computer generated perspective drawing of the final X-ray model less hydrogens. Additional crystallographic details may be obtained from Professor J. Clardy. In general the metric details agree well with generally accepted values. There were no abnormally short intermolecular contacts or unusually high electron density in a final difference synthesis.

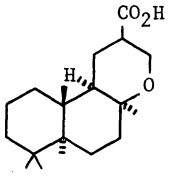
Figure 1 presents a drawing of the X-ray model of the ozonolysis product of puupehenone less hydrogens. The X-ray experiment did not define the absolute configuration so the enantiomer shown represents an arbitrary choice. The AB ring junction is trans with the methyl at C(10) on the opposite side from the hydrogen at C(5). The BC ring junction is cis with both substituents α. The nine atom fragment composed of C(9), C(10), C(11), C(13), C(17), O(22), O(23), and O(24) is essentially planar with the largest deviation from the best least squares plane being 0.16 Å. The A and B rings have the expected chair conformation.

A second but minor product, a colorless solid, insoluble in organic solvents, but soluble in sodium bicarbonate with effervescence, was also isolated from the ozonolysis. It was characterized by mass spectrometry (m/e 102, 58, 44, 29, 28) and is therefore most likely oxomalonic acid semialdehyde, $C_3H_2O_4$ ($\underset{\sim}{2}$).



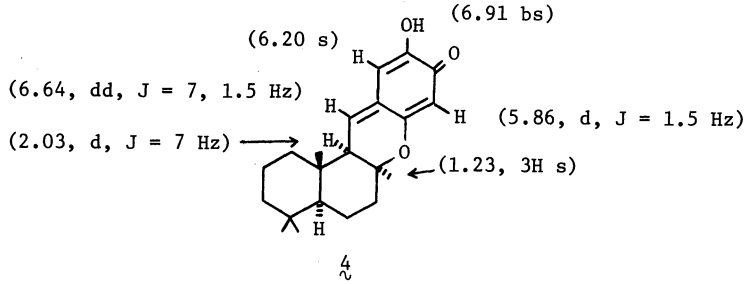
$\underset{\sim}{2}$

Compound $\underset{\sim}{1}$ was further characterized as an orange, 2,4-DNP derivative, mp 135-137° (dec), which has its aldehydic proton signal shifted to δ 8.25 ppm; and secondly by Jones oxidation as a noncrystalline carboxylic acid, $C_{18}H_{26}O_4$. This acid underwent anomalous LAH reduction and resulted, surprisingly, in a saturated acid to which structure $\underset{\sim}{3}$ was assigned on the basis of spectral data.



$\underset{\sim}{3}$

The result of the ozonolysis reaction and the X-ray data of $\underset{\sim}{1}$ allow us to formulate puupehenone as $\underset{\sim}{4}$. All spectral data (vide supra) are in full accord with this structure, in



$\underset{\sim}{4}$

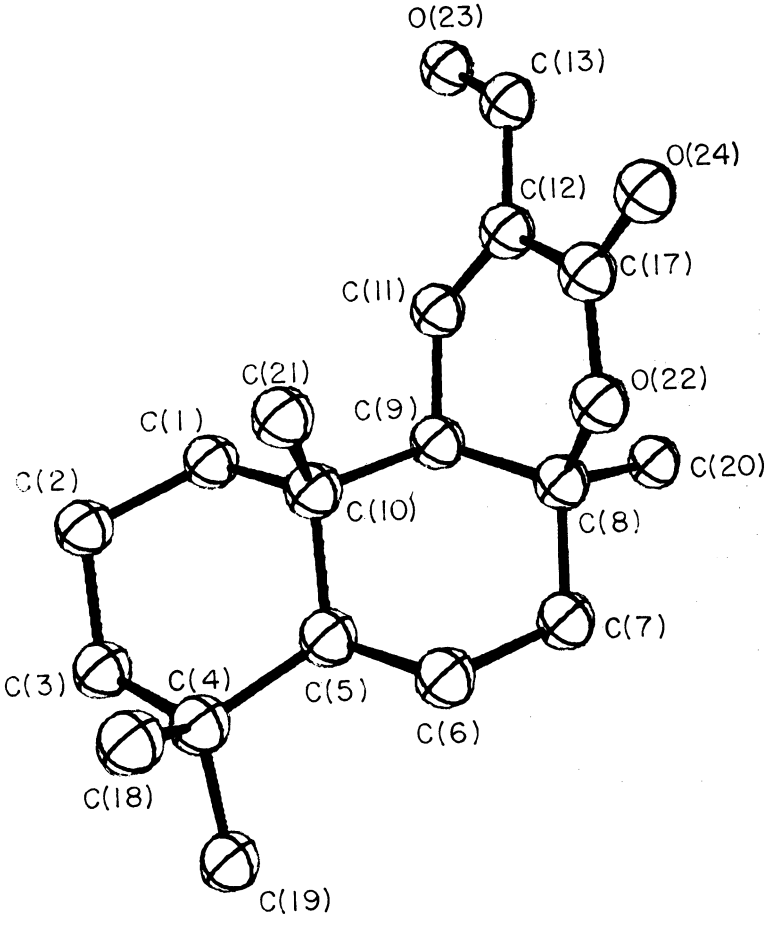
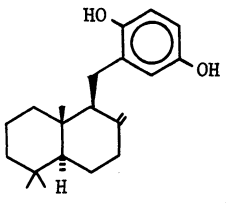


Figure 1. A computer generated drawing of the ozonolysis product of puupehenone. Hydrogens are omitted for clarity and the X-ray experiment did not define the absolute stereochemistry.

which the proton chemical shift assignments of the functionalized part of 4 are shown. Puupehenone thus belongs to a group of metabolites, in which a sesquiterpene moiety is linked to a quinone or a quinol. Marine representatives of this class of compounds are, inter alia, zonarol, (5) from the brown alga Dictyopteris zonariodes (12), the sponge



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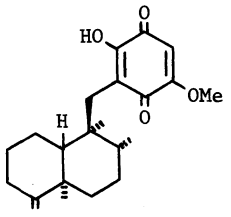

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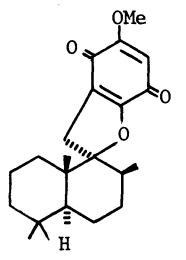

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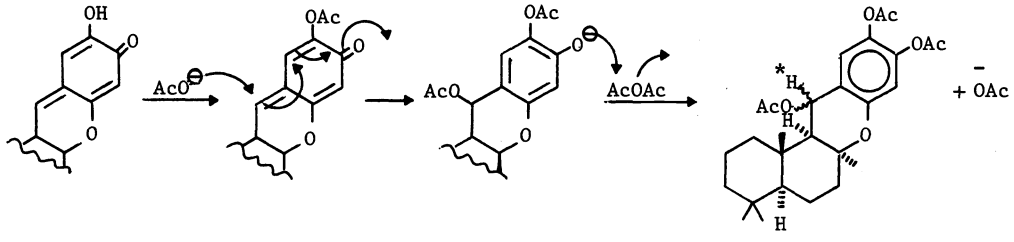
metabolites ilimaquinone (6) (13), or cyclospongiaquinone-2 (7) (14). A total synthesis of (±)-puupehenone (4) has been described by Trammell (15), and the compound is undergoing anti-tumor testing at the National Cancer Institute (16).

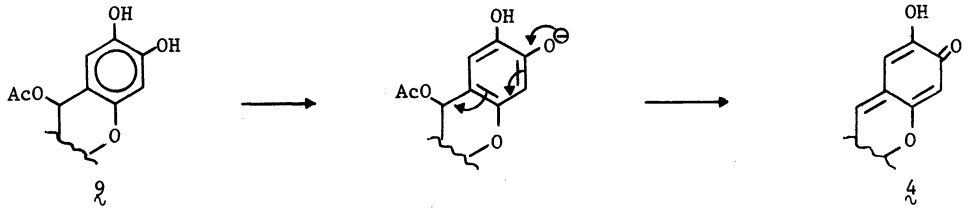
Among our early attempts at a better definition of the oxygen functions of puupehenone (4) was a routine acetylation reaction which we expected to yield an enol monoacetate. Instead, we isolated a triacetate with a radically changed rotation, $[\alpha]_D$ -89° (c 0.975, CCl_4), and a uv spectrum of an aromatic compound, 285 (3,500), 291 (3,400) nm. The ^1H nmr spectrum in deuteriochloroform displayed three low field one proton singlets at δ 7.06, 6.66, and 6.02 ppm and three acetate methyls -- two as a six proton singlet at δ 2.24 and one three proton singlet at δ 2.05 ppm. The acetate signals are complemented by ir bands at 1780 (vinylic) and 1740 cm^{-1} (normal), ascribed to acetates. Further clues that more than routine acetylation had taken place came from mass spectral data. The expected composition of a puupehenone triacetate is $C_{21}H_{28}O_3 + 3(CH_2CO)$ or $C_{27}H_{34}O_6$. The experimental data, however, proved that the composition of the triacetate was $C_{27}H_{36}O_7$ with a molecular weight of 472 daltons or greater than anticipated by 18 mass units. Since no water was introduced during the acetylation reaction or during work-up, the elements of water must be derived from acetic anhydride. This information allows us to formulate a plausible reaction sequence leading to puupehenone triacetate (8). This formulation was confirmed by palladium on carbon



4 8

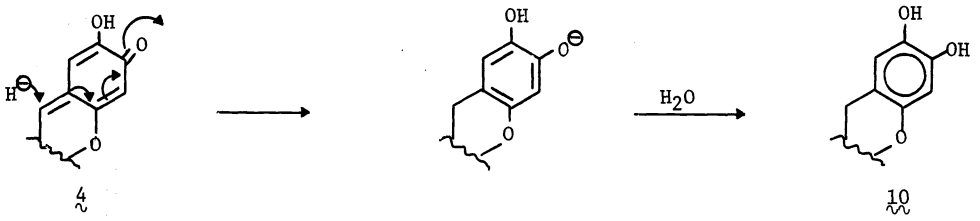
hydrogenation of 8. This reaction led to a diacetate, mp 119-120°, which lacks the 1780 cm^{-1} infrared band of 8 as well as its three proton singlet at δ 2.05 ppm. The benzyl proton (asterisked in 8) which resonates at δ 6.02 ppm is replaced in the diacetate by a poorly resolved multiplet centered at δ 2.85 ppm.

In another interesting reaction of the triacetate, treatment of 8 with lithium aluminum hydride in ether, puupehenone (4) is regenerated. If we assume a catechol (9) to be the proximate reduction product, elimination of the benzylic acetate in basic medium can be rationalized.



9 4

Another aromatization of puupehenone (4), again surprising at first, takes place upon reaction with sodium borohydride in methanol, which adds two hydrogen atoms in the following fashion. Catechol 10 forms two isomeric guaiacols and a veratrol upon treatment with diazomethane. Acetylation of 10 leads to a normal diacetate, identical with the hydrogenolysis product of triacetate 8.

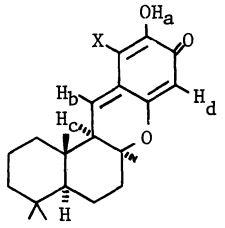


4 10

BROMO- AND CHLOROPUUPEHENONE

A yellow encrusting sponge that turns brown on exposure to air was collected at Enewetak Atoll, Marshall islands (17). The freeze-dried animal was thoroughly extracted with dichloromethane. The concentrate of this extract was chromatographed on Florisil. Elution was carried out with petroleum ether, petroleum ether-benzene, benzene, followed by chloroform-benzene mixtures, and eventually chloroform. The benzene eluates (5 ℓ) contained all of the organic compounds. Fractions 9-13 after additional chromatography on Bio-Sil A and Sephadex LH-20 furnished a colorless compound, mp 234-240°, $C_{42}H_{54}O_6$. Fractions 16-24 yielded puupehenone (4), which could be crystallized from hexane, mp 129-130°, $C_{21}H_{28}O_3$. Fractions 27-39 of the primary Florisil chromatography when rechromatographed on Sephadex LH-20 gave rise to a yellow compound, mp 335° (dec), $C_{42}H_{52}O_6$, and to an oil which after Bio-Sil A chromatography became a yellow glass, the mixture of halopuupehenones. In subsequent work-ups the methylene chloride extract of the freeze-dried sponge was first chromatographed on Bio-Sil A, followed by preparative tlc on silica gel (4% MeOH/$CHCl_3$), which provided fairly clean separation into a yellow glass (R_f 0.5), which are the halo derivatives, and puupehenone (4), R_f = 0.6.

The predominant halo derivative, chloropuupehenone (11, X = Cl), could be further purified by additional preparative tlc on silica gel. The mass spectrum of 11, $C_{21}H_{27}ClO_3$, matched that of puupehenone (4, m/e 328, base peak m/e 177) except for additional peaks at m/e 364 and 362 in the ratio of 1:2.5 and a shifted base peak at m/e 211. Its structure was deduced from



11 X = Cl
12 X = Br

comparison of its ^1H nmr spectrum with that of puupehenone (4). Pertinent chemical shifts are summarized in Table 2. Double resonance experiments in deuteriobenzene further

TABLE 2. Comparison of ^1H Nmr Spectral Data of Puupehenone (4) and Chloropuupehenone (11) in $CDCl_3$ (δ units)

Proton	11	4
H_a	7.17 (1H, br s)	6.91 (1H, br s)
H_b	7.14 (1H, dd, J = 7, 2 Hz)	6.64 (1H, dd, J = 7, 1.5 Hz)
H_c	2.16 (1H d, J = 7 Hz)	2.03 (1H, d, J = 7 Hz)
H_d	5.84 (1H d, J = 2 Hz)	5.86 (1H, d, J = 1.5 Hz)

substantiated these assignments. Missing, of course, in 11 is the singlet signal of puupehenone (4) at δ 6.20 ppm.

Mass spectra of chromatographic fractions had exhibited weak molecular ion peaks of equal intensity at m/e 408 and 406 and composition of $C_{21}H_{27}BrO_3$, thereby indicating presence of a monobromo derivative of puupehenone (4). The reasonable assumption that the bromo compound had structure 12 was difficult to demonstrate because of our inability to separate a pure compound, by Sephadex chromatography, by tlc, or by HPLC on a C_{18} reversed phase column. We eventually resorted to derivatization prior to separation. We treated the mixed puupehenones with acetic anhydride and 4-N,N-dimethylaminopyridine and obtained after repeated preparative tlc the well-characterized puupehenone triacetate (8) and the mixed halo derivatives as a colorless glass. This mixture was separable by C_{18} reversed phase tlc in 10% aqueous acetonitrile into the major constituent (R_f 0.4) 11 and the minor bromo compound, R_f 0.3, as a colorless oil. Its principal ^1H nmr signals ($CDCl_3$) are compared with those of

chloropuupehenone triacetate and of 8 in Table 3. This comparison, bolstered by calculated ^{1}H nmr data, proves that bromopuupehenone does indeed possess structure 12. The calculated shift of the aromatic proton (18) in 12 is 6.58 ppm, in fair agreement with the observed

TABLE 3. Principal ^{1}H Nmr Data of the Puupehenone Triacetates ($CDCl_3$, δ)

Proton	Bromo	Chloro	8
Aromatic	6.68 1H s	6.69 1H s	7.06, 6.66, 1H s each
Benzylic	6.02 1H bs	6.04 1H bs	6.02 1H s
Acetate	2.32 3H s	2.34	2.24
do.	2.27 3H s	2.28	2.24
do.	2.03 3H s	2.04	2.05
Methyl	1.30 3H s	1.31	1.26
do.	0.88 3H s	0.89	0.90
do.	0.80 3H s	0.80	0.82
do.	0.63 3H s	0.64	0.64

value of 6.68 ppm. If one performs the calculation for the only alternate structure that places the bromine atom peri to the ether oxygen, one arrives at δ 6.92 ppm, which differs by more than 0.3 ppm from the observed value.

Since we had discovered (vide supra) that lithium aluminum hydride reduction of puupehenone triacetate (8) regenerates puupehenone (4), we attempted this route in order to obtain spectral data of pure bromopuupehenone (12). As our supply of chloropuupehenone triacetate was more plentiful, we ran the reaction on 10 mg of this compound and achieved a 40% yield of 11 without loss of chlorine. Encouraged by this result we subjected 2.2 mg of bromo-puupehenone triacetate to lithium aluminum hydride reduction. In that case, however, the reaction product, 1.5 mg of yellow oil, proved to be a complex mixture as judged by ^{1}H nmr data.

The three puupehenones (4, 11, 12) are constructed of a sesquiterpene moiety, which has a normal drimane skeleton analogous e.g. to the algal metabolite zonarol (5), and of a 1,2,4-trihydroxybenzene derivative. If the biogenetic precursor of the aromatic moiety is the corresponding 2,3,5-trihydroxybenzoic acid, introduction of halogen might occur concomitant with decarboxylation and thus lead to the halopuupehenones 11 and 12. It is interesting to speculate why in this case -- in reversal of the common pattern among marine metabolites -- the chloro derivative (11) predominates over the bromo compound (12).

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