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NOVEL BIOACTIVE DITERPENOID METABOLITES FROM TROPICAL MARINE ALGAE OF THE GENUS *HALIMEDA* (CHLOROPHYTA)

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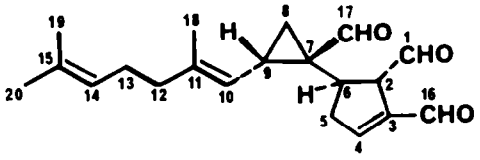
Abstract--Four new diterpenoid metabolites, halimedatrial (**1**), **3**, **4** and **5**, have been isolated from several species of the tropical green algae *Halimeda* (Udoteaceae). These new compounds show potent antimicrobial and cytotoxic properties in laboratory bioassays.

Marine algae of the family Udoteaceae (Chlorophyta) are among the most abundant and widely-distributed seaweeds in tropical and subtropical habitats. Although grazing intensity due to herbivorous fishes and invertebrates is generally high in tropical reef environments, feeding preference studies and stomach content analyses have shown that algae of the family Udoteaceae are among the least preferred of the available food items.[1-5] As part of our continuing interest in the chemical adaptations of tropical marine organisms, we and others have recently reported investigations of the natural products chemistry of representatives of this group involving the genera *Rhipocephalus*,[6] *Tydemania*,[7] *Chlorodesmis*,[8] *Avrainvillea*,[9], *Udotea*,[10-13] and *Penicillus*.[13] Of the representatives within this group, those of the genus *Halimeda* are the most widely distributed and generally found in abundance in areas of high predator activity. *Halimeda* species attain rather high levels of calcification (up to *ca.* 80% $CaCO_3$), and the coarse and unpalatable textures which result have been proposed as a major physical adaptation against predation. In a recent paper, we reported our initial findings of the presence of a highly bioactive terpenoid, halimedatrial (**1**), in extracts of numerous *Halimeda* species.[14] The potent fish and larval toxicities, as well as the feeding deterrence effects produced by this metabolite, prompted us to suggest that halimedatrial represents the basis for a chemical defense adaptation in this important pantropical genus.

In this paper we wish to report, in detail, upon the complete natural products chemistry of 12 species of algae of the genus *Halimeda* collected in both the tropical Atlantic and Pacific Oceans. In addition to halimedatrial (**1**), three new metabolites, **3-5**, have been isolated and structurally defined. These new compounds, like those previously reported, possess antimicrobial and cytotoxic properties.

Over a two year period, over 50 collections of 12 different *Halimeda* species were obtained and chemically defined. Table 1 lists the species collected, the sites of collection and the average chemical compositions observed. It is noteworthy that compounds **1** and **4** are the major metabolites found and that little variation was noted between Atlantic and Pacific Ocean collections. Contrary to our earlier report based on fewer investigations, halimedatrial does not appear to be *the* major metabolite in all species studied. Halimedatrial is usually a major metabolite, however, and it is the major terpenoid in several species.

The structure of halimedatrial (**1**), was presented briefly in an earlier paper,[14] and it is included here for comparative purposes and for completeness. As briefly described earlier, the structure of halimedatrial (**1**) was assigned on the basis of its spectral properties and its conversion to the triacetate **2** with sodium borohydride in methanol followed by acetylation. Proton and ^{13}C NMR data for halimedatrial, and for the new compounds **3-5**, are presented in Tables 2-4. Halimedatrial analyzed for $C_{20}H_{26}O_3$ by combined spectral methods, which indicated 8 degrees of unsaturation. Although not



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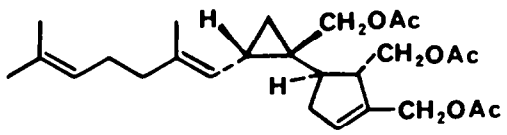

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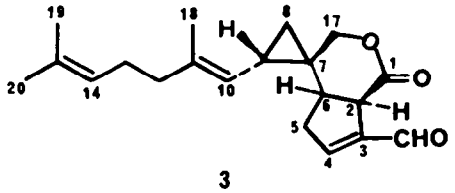

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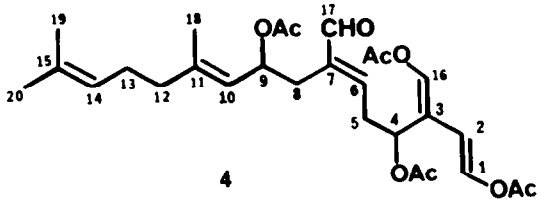

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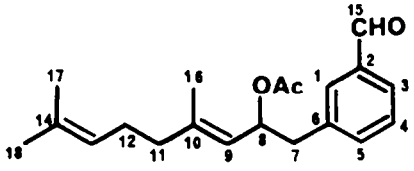


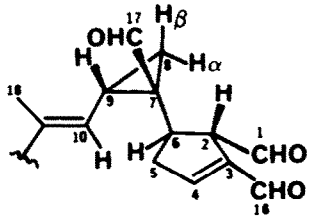
5

apparent in the spectral data, a cyclopropane ring was ultimately recognized to be present by ^{13}C NMR features which showed the large natural coupling constants for the cyclopropane carbons C-8 and C-9 (Table 3).[15] Other unsaturations in **1** consisted of three aldehyde carbonyls and three double bonds. Hence, halimedatrial was concluded to be a bicarbocyclic diterpenoid. Intense mass spectral fragmentation of a $C_{10}H_{15}$ unit (135.1172), in combination with the well-known terpenoid ^1H and ^{13}C NMR spectral features,[15] showed that **1** possessed a linear terpenoid fragment entirely analogous to geraniol. This fragment was conceived to result from cleavage of the cyclopropane ring at C-7 and C-8.

Further assignments of the substructure of halimedatrial could be made by ^1H NMR decoupling studies. The protons at C-2, C-4, C-5 and C-6 were readily inter-related which defined the presence of the substituted cyclopentene ring. Initially, some confusion existed regarding the presence of three aldehyde groups. In two solvents ($CDCl_3$ and Bz-d_6), only two ^{13}C doublets at low field were visible (200.2 and 188.5 ppm). The ^1H NMR shift of one of the aldehyde protons (δ 8.57, s) was also misleading. Conversion of **1** to the corresponding triol and then to the triacetate **2** provided a solution to these problems. The spectral details for **2** (experimental), clearly showed the presence of three primary acetates and also delineated, more clearly, the presence of the cyclopropane and cyclopentene rings. Complete spectral analysis and assignment of this derivative was essential for the confident structural assignment of **1**.

Although coupling constant analysis was possible, the similar coupling constants between *cis* and *trans* protons in both the cyclopropane and cyclopentene rings precluded assignments of the relative stereochemistry of **1** at its 4 asymmetric centers. Stereochemical information was, however, readily obtained from ^1H nuclear Overhauser enhancement experiments involving spectral subtraction techniques (Difference NOE). Table 4 summarizes these results (see partial structure a). Intense enhancements of the "up" or β C-8 proton and the C-9 proton were observed when the C-17 aldehyde proton was irradiated. This experiment fixed the aldehyde and C-9 proton on the same "β" face of the cyclopropane ring. Strong enhancement of the C-6 proton was also observed when the C-10 olefinic proton was irradiated. This indicated that these protons were in spatial proximity beneath the cyclopropane ring. Since C-6 was assigned as the ring juncture methine proton, it was concluded to be "down" (or α) relative to the plane of the cyclopentene ring. The stereochemistry at C-2 was fixed, but with

lesser confidence, by consideration of the overall stereochemical features of the molecule. Irradiation of the C-2 proton showed enhancements of the 4 protons, C-1, C-6, C-8α and C-17. Molecular models clearly showed that these enhancements are much more likely when the C-2 proton is in the "up" or β position.



a

Halimedatrial is structurally related to udoteatrial (**6**) a hydrated trialdehyde recently described by

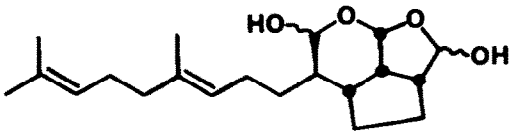


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Table 1

Chemical Composition of *Halimeda* Species[a,b]

Alga	Collecting Site(s)	metabolites 1	3	4	5
H. incrassata	Puerto Rico, Bahamas, Florida Keys	31%, 1200 ppm	--	25%, 800 ppm	--
H. monile	Bahamas	10% 600 ppm	--	70%, 5000 ppm	--
H. opuntia	Puerto Rico, Bahamas, Florida Keys	20%, 900 ppm	--	20%, 900 ppm	--
H. copiosa	Bahamas, Florida Keys	10%	--	15%	--
H. tuna	Bahamas, Florida Keys, Pacific Mexico	20%	5%	25%	--
H. discoidea	Puerto Rico, Florida Keys	--	--	25%	--
H. goreauii	Bahamas, Florida Keys	--	--	5%	--
H. cylindracea	Saipan	10%	--	--	--
H. incrassata	Guam	--	--	30%, 900 ppm	--
H. discoidea	Guam, Hawaii	--	--	25%, 2500-8000 ppm	2%, 200 ppm
H. macroloba	Guam	--	--	80%, 9600 ppm	3%, 300 ppm
H. opuntia	Guam - several collections	10%, 500 ppm	--	20%, 1000 ppm	--
H. gigas	Guam	10%, 500 ppm	--	15%, 800 ppm	--
H. scabra	Bahamas	10%	4%	20%	10%
H. simulans	Bahamas	25%	--	--	--

a - values reported in percent are concentrations of metabolites in percent of the organic extract

b - values reported as ppm are concentrations of metabolites as parts per million of the algal dry weight

Table 2

^{1}H NMR Spectral Data for *Halimeda* Terpenoids[a,b]

C#	1	3	4	5
1	10.0, d, J=1 Hz	--	7.65, d, J=12.7 Hz	7.73, s
2	4.18, m	4.17, m	5.85, d, J=12.7 Hz	--
3	--	--	--	7.73, m
4	6.96, d, J=2 Hz	7.04, d, J=2 Hz	6.00, t, J=7 Hz	7.48, m
5	2.70	α-2.62, m β-2.91, m	3.05, m 2.76, m	7.48, m
6	2.46, m	1.72, m	6.47, t, J=7 Hz	--
7	--	--	--	3.05, dd, J=13.3,6.7 2.85, dd, J=13.3,6.8
8 α β	1.23, dd, J=6,5 Hz 1.55, dd, J=9,6 Hz	0.70, ddd, J=6,5,2 Hz, 1.25, dd, J=9,5 Hz	2.57, d, J=7 Hz	5.67, ddd, J=9.2,6.8, 6.7 Hz
9	2.22, ddd, J=9,8,5 Hz	1.65, m	5.53, dd, J=8,7 Hz	5.14, d, J=9.2 Hz
10	4.99, d, J=8 Hz	4.77, d, J=8.5 Hz	5.11, d, J=8 Hz	--
11	--	--	--	1.98, m
12	2.08, m	2.05, m	2.04, m	1.98, m
13	2.08, m	2.05, m	2.04, m	5.02, t, J=6 Hz
14	5.04, m	5.06, t, J=6.5 Hz	5.04, m	--
15	--	--	--	9.99, s
16	9.77, s	9.79, s	7.29, s	1.51,[a] s
17[d] a b	8.57, s	3.38, dd, J=11.5,2 Hz 4.68, dd, J=11.5,2 Hz	9.35, s	1.58,[c] s
18	1.60,[c] s	1.60,[c] s	[a]1.59, s	1.67,[c] s
19	1.65,[c] s	1.67,[c] s	[a]1.67, s	--
20	1.72,[c] s	[a]1.70,[c] s	1.67,[c] s	--
Acetates	--		2.19, s 2.17, s 2.09, s 1.97, s	1.99, s

a - Recorded at 360 MHz in $CDCl_3$ solution.

b - Abbreviations: s, singlet; d, doublet; t, triplet, m, multiplet; α notations refer to protons below the plane of the molecule, β notations refer to protons above the plane.

c - Values may be switched.

d - 17a - proton on same side of lactone ring as cyclopropane.
17b - proton anti with respect to cyclopropane.

the Faulkner group.[10] Udoteatrial exists exclusively as the hydrate, or cyclic acetal-bis-hemiacetal, and the unhydrated trialdehyde has never been observed. All attempts to reproduce this reactivity by hydrating halimedatrial were unsuccessful. Re-evaluation of the proposed stereochemistry of **1** showed that intramolecular cyclization could only be expected when the stereochemistry at C-2 and C-6 was *cis*. This structure factor is evident in udoteatrial, but in halimedatrial (**1**) the C-2-C-6 stereochemistry is predicted to be *trans* on the basis of NOE measurements. Cyclization of **1** as in udoteatrial would produce a *trans* oxo-hydrindane which is predicted to possess significant ring strain.

A minor metabolite, assigned as halimedalactone (**3**), was isolated from several collections of *H. tuna* and *H. scabra*. This new compound analyzed for $C_{20}H_{26}O_3$ by HRMS (M^+ m/z = 314.1854), and this formula was sustained by its ^{13}C NMR features (Table 3). There were very obvious spectral similarities between **3** and halimedatrial. The presence of a cyclopropane ring was concluded on the basis of ^{13}C NMR bands at 21.8 (t) and 19.8 (d), both of which possessed large natural couplings.[15] The α,β-unsaturated aldehyde was also intact, as could be concluded from infrared data (1690, 1600 cm^{-1})

and from ^{1}H and ^{13}C bands which were quite analogous to those from halimedatrial. In compound **3**, however, two of the oxidized methyl vestiges had obviously been converted to a lactone. This structural feature was accompanied by intense infrared absorption at 1730 cm^{-1} and by ^{13}C NMR bands at 169.6 (s) and 72.7 (t) ppm. As in halimedatrial, intense fragmentation of $C_{10}H_{13}$ (133.1005), coupled with appropriate ^{13}C NMR bands, showed that the terpene chain past C-9 was unsubstituted. The methyl group ^{13}C NMR shifts observed for **3** also illustrated the C-9-C-10 olefin to be in the *E* configuration.[15]

The final structure assignment of halimedalactone was made by ^{1}H NMR decoupling experiments which allowed all protons to be assigned (Table 2, partial structure b). A small "W" coupling between the C-8α proton and the C-17$_b$ proton *anti*-oriented to the cyclopropane ring confirmed the placement of the lactone carbonyl group at C-1. An additional "W" coupling (~2 Hz) was observed between the C-17$_a$ proton *syn*-oriented to the cyclopropane ring and the down or α-oriented proton at C-6. Again, NOE measurements were used to finally assign the overall relative stereochemistry of halimedalactone. Enhancement of one of the C-17 protons, on irradiation at C-9, showed their *cis* relationship on the cyclopropane ring. Irradiation of the C-10 olefin proton produced enhancements of the protons at C-6 and C-8α. Irradiation at C-2 produced enhancements at C-6, and contrary to halimedatrial, also

Table 3

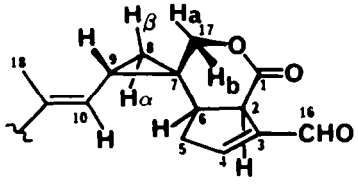
^{13}C NMR Spectral Data for *Halimeda* Terpenoids[a]

C#	1	$J_{C\text{-}H}$	3	$J_{C\text{-}H}$	4	5
1	[d]201.2 d	174	169.6 s	..	[f]137.1 d	130.9 d
2	60.2 dd	135, 25	48.9 d	141	108.7 d	138.7 s
3	131.3 s		131.7 s		118.1 s	128.4 d
4	155.7 d	166	152.4 d	161	[c]67.9 d	129.1 d
5	37.4 t	135	37.8 t	132	[b]32.5 t	136.0 d
6	39.1 d	130	41.1 d	136	149.4 d	141.6 s
7	40.2 s		[c]27.4 s		131.6 s	41.3 t
8	20.5 t	163	21.8 t	162	[b]29.5 t	71.7 d
9	24.5 d	161	19.8 d	157	[c]69.9 t	123.9 d
10	[b]124.7 d	151	[d]123.8 d	145	[d]123.7 d	136.7 s
11	[c]145.0 s		[b]142.3 s		[e]141.0 s	39.7 t
12	39.9 t	125	39.6 t	133	39.4 t	26.6 t
13	26.9 t	125	[c]26.4 t	126	26.2 t	122.4 d
14	[b]120.8 d	156	[d]121.5 d	158	[d]122.5 d	132.0 s
15	[c]141.3 s		[b]138.8 s		[e]141.3 s	192.5 d
16	189.6 d	177	187.4 d	180	[f]134.4 d	17.0 q
17	[d]200.0 d	178	72.7 t	157	193.7 d	17.9 q
18	16.9 q	130	16.6 q		16.8 q	25.9 q
19	17.7 q	126	17.7 q		17.6 q	..
20	25.8 q	125	25.7 q		25.6 q	..
Acetates					169.9 s 167.7 s 167.6 s 166.7s 21.2 q 20.8 q 20.6 q 20.6 q	170.4 s 21.5 q

a - Recorded at 50 MHz in Me_2CO-d_6 (for 1), $CDCl_3$ (for 3 and 4) and Bz-d_6 (for 5) solutions with TMS as internal standard. $J_{C\text{-}H}$ values (natural couplings) are in Hertz and were determined by gated decoupling techniques.

b,c,d,e,f - Values may be interchanged

enhanced the aldehyde proton at C-16. This latter difference suggested that the stereochemistry at C-2 was inverted in **3** relative to halimedatrial. Indeed, as earlier discussed, the lactone cyclization would be unlikely in the C-2 - C-6 *trans* configuration due to extreme ring strain. On these grounds we suggest halimedalactone has the inverted stereochemistry at C-2 and gross structure illustrated in *3*. Halimedalactone showed $[\alpha]_D \simeq 0^0$, which could be interpreted to suggest either a racemic mixture or most likely that the rotation is fortuitously low at the sodium-D line.



b

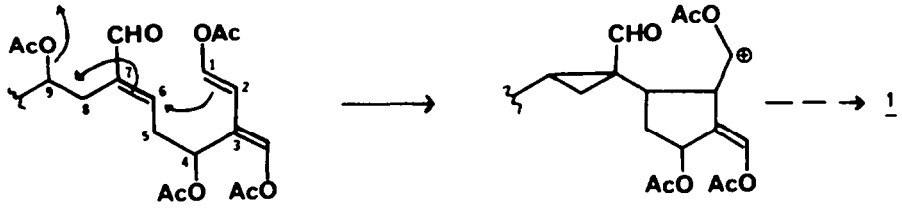
Given the abundance of enol-acetate containing linear terpenoids in related algae within the family Udoteaceae,[6,8,11,13] we were not surprised to isolate the tetra-acetate **4** as a major metabolite in numerous *Halimeda* species. Diterpenoid **4** analyzed for $C_{28}H_{38}O_9$ by HRMS (M^+-HOAc, m/z = 458.2302) and also by interpretation of ^{13}C NMR data. Spectral features (^1H and ^{13}C NMR) for the well-known bis-enol-acetate constellation were easily assigned, but in particular the characteristic ^1H bands at δ 7.65 (d, J = 12.7 Hz), δ 7.28 (s), and δ 5.85 (d, J = 12.7 Hz) were most obvious in this metabolite. NOE measurements of the enhancement of the C-2 and C-16 proton, as reported earlier, fully defined this terminal diene constellation as *E, E* (Table 4).[11]

In addition to the terminal bis-enol-acetate, **4** was recognized by combined spectra features to possess two secondary acetate esters and an α,β-unsaturated aldehyde group. Two additional methyl-trisubstituted double bonds were present (Tables 2 and 3), which fully accounted for the 10 degrees of unsaturation in the molecule. Hence, **4** was concluded to be a highly oxidized linear diterpenoid.

Proton NMR experiments with metabolite **4** allowed the structure to be confidently assigned and all protons to be identified (Table 2). In particular, the sites of oxidation could be assigned between C-4 and C-9 on the basis of ^1H NMR decoupling results. The secondary acetate at C-4 was readily assigned by comparison with other related terpenoids,[6,13] and the C-4 methine proton was determined to be coupled to the C-5 methylene pair. These protons were, in turn, determined to be coupled to the C-6 proton. The low field position of the C-6 proton confirmed it as the β proton of the unsaturated aldehyde. The geometry of the C-6-C-7 olefin was concluded to be *E* on the basis of the chemical shift for the aldehyde proton.[16,17]

The positioning of the secondary acetate at C-9 in this compound resulted from several decoupling experiments. First, the C-14 proton was defined since it was allylically coupled to both the C-19 and C-20 methyl groups. The C-14 olefin proton was observed as a typical triplet coupled to the C-13 methylene protons, which were also typical and found at δ 2.04 as a complex multiplet. Hence, decoupling experiments defined a typical terminal isoprenoid unit void of oxygenation. The second acetate could easily be positioned at C-9, also on the basis of decoupling experiments. The olefinic proton at C-10 was found to be coupled to the C-9 proton, which was itself coupled to the methylene protons at C-8. The complete structure of **4** was then secured, void however of stereochemistry at both C-4 and C-9.

A clear structural relationship exists between the tetra-acetate **4** and halimedatrial (**1**). A direct conversion of **4** to **1** can be envisioned by a combination of several mechanisms involving solvolysis and hydrolysis. Solvolysis of the C-9 acetoxyl group would produce a homoallylic carbonium ion which could be trapped by the C-6-C-7 olefin to produce a cyclopropyl carbinyl cation at C-6. Cyclization of this carbonium ion to C-2 yields the 5 membered ring component of halimedatrial, and after elimination of the allylic acetoxyl at C-4 and hydrolysis, halimedatrial itself.



Since this latter interconversion was conceivable and suggested halimedatrial could be produced during isolation, the reactivity of the tetra-acetate **4** was explored. Compound **4** was subjected to strong hydrolytic conditions employing both acid and base catalysis (p-tosylic acid/aq Me_2CO; KOH/MeOH; BF_3 etherate) under a variety of temperatures and reaction times. Under no circumstances could the tetra-acetate be induced to form **1**. Generally **4** was stable toward these mild reagents and was found to decompose under stronger conditions.

Although it is unlikely that **4** is chemically converted to **1** (spontaneously or upon workup), a close biosynthetic relationship clearly exists between these two compounds. Analysis of numerous individual *Halimeda* plants showed that **4** and **1** regularly co-occur.

Another minor compound we have isolated from two *Halimeda* species from Guam (*H. macroloba* and *H. discoidea*), and *H. scabra* from the Bahamas, is the unusual bis-nor diterpenoid **5**. This compound analyzed for $C_{20}H_{26}O_3$ by combined spectral methods and was recognized as a C_{15} terpenoid after the presence of an acetate ester was confirmed. Spectral features for **5** showed the presence of a *meta*-substituted benzaldehyde group. These features included benzenoid UV absorption at 286 nm

Table 4

Results of ^1H NMR NOE Experiments[a,b,c]

Irradiation of Protons on Carbon #	Compounds, Protons Enhanced		
	1	3	4
1	2,6	..	
2	1,6,8α,17	5α,6,16	16
3	..	..	
4	5,16	16	
5	4,6	2,4,6	
6	1,2,5,8α,10	2,10	
7	..	..	
8α	2,6,8β,10	8β,6,10	
8β	8α,9	8α,9,17a	
9	8β,17	8β,17a	
10	6,8α,12	5,6,8α	
11			
12			
13			
14			
15			
16	4		2
17	9	8β,9,17	
18			
19			
20			

a - Experiments performed by nuclear Overhauser enhancement difference spectral methods (NOEDS) at 360 MHz in degassed $CDCl_3$ and $Br\text{-}d_6$ solution

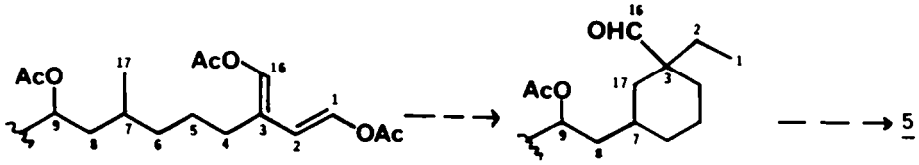
b - α and β notations refer to protons below and above the general plane of the molecule

c - The C-17 protons have been defined as a and b since they cannot be designated as "up or down" relative to the cyclopentene and cyclopropane rings. The C-17a proton refers to the proton *syn* to the cyclopropane ring, whereas the C-17b designation refers to the *anti*-oriented proton

(3500) and 249 nm (6700) and four proton NMR signals showing typical couplings and shifts for this classical constellation.[18] ^{13}C NMR values for the aromatic portion of this metabolite were also in accord with predicted values.[19]

The mass spectrum of **5** showed an intense peak (the base peak, 135.1175) for fragmentation of a $C_{10}H_{15}$ hydrocarbon unit. This feature, in combination with the linear terpene nature of all these metabolites, showed that all oxygenation was on one end of the molecule. Intense mass spectral benzylic cleavage of the C-7-C-8 bond was also observed (50% base, 119.0508) yielding an aromatic fragment which analyzed for C_8H_7O. Proton NMR experiments were conclusive in assigning the final structure of **5**. The connectivity of C-7-C-8-C-9 was readily observed by decoupling experiments and the allylic coupling of the C-9 olefin proton to one methyl group (C-16) was also clear. In addition, allylic coupling of the C-18 olefin proton to both the C-17 and C-18 methyls served to define and differentiate these protons.

The biogenetic origin of this bis-nor diterpenoid is unclear. It is conceivable that cyclization of an appropriate enol-acetate precursor between a methyl carbon (C-17) and C-3 could generate a cyclohexane system which could then lose a C_2 unit through aromatization.



The four new metabolites described above possess a variety of biological activities. They exhibit antimicrobial activities against marine and terrestrial bacteria and fungi, and they inhibit cell division of fertilized sea urchin eggs at or below 16 μg/ml. Complete details of the biological assays involved and the results obtained will be published elsewhere.

EXPERIMENTAL

General: IR spectra were recorded on a Perkin-Elmer model 137 spectrophotometer and UV spectra were obtained in MeOH on a Beckman Mk IV instrument. Proton NMR spectra were obtained on a 360 MHz Nicolet-Oxford Magnetics FT spectrometer and ^{13}C NMR spectra were recorded at 50 MHz on a Nicolet NT-200 instrument. High resolution mass spectra were obtained through the Mass Spectrometry Resource Center, School of Pharmacy, UC San Francisco. Algae were collected in the Bahama Islands (September 1981, July 1982, July 1983), the Florida Keys (November 1983), and Guam (April-June 1983). The freshly collected plants were immediately macerated and extracted twice with CH_2Cl_2. The extracts were condensed to dark residues and immediately chromatographed on silica gel with CH_2Cl_2-MeOH solvent mixtures. If either frozen algae or crude extracts were allowed to stand or shipped prior to chemical analysis, significant decomposition was noted in almost all cases. Final purification of compounds was accomplished by preliminary Florisil chromatography (for removal of pigments) followed by preparative silica HPLC with EtOAc/isooctane solvent mixtures.

Halimedatrial (**1**). Table 1 indicates the species of *Halimeda* investigated which were found to contain halimedatrial. Halimedatrial was first isolated from *H. tuna* as a yellow oil (20% of the organic extract) after silica gel HPLC (40% EtOAc/isooctane). Halimedatrial showed $[\alpha]_D^{25} = -59^0$ (c = 0.9, $CHCl_3$); IR ($CHCl_3$) 2940, 1720, 1690, 1675, 1610, 1450, 1375 cm^{-1}; UV (MeOH) λ_{max} 236, ϵ = 11,400; HRMS M^+ found 314.1864, calc. 314.1875 ^{13}C NMR (50 MHz, $CDCl_3$): 2 x 200.2 (d), 188.5 (d), 154.4 (d), 144.1 (s), 142.0 (s), 131.7 (s), 123.6 (d), 119.1 (d), 59.8 (d), 40.5 (s), 39.3 (t), 37.5 (d), 36.4 (t), 26.2 (t), 25.6 (q), 24.1 (d), 20.2 (t), 17.6 (q), 16.8 (q).

Triacetate **2**. Halimedatrial (50 mgs, 0.16 mmoles) in 5 ml MeOH was treated with 1.5 eq. (9 mg) sodium borohydride at 0^0 for 15 min and the mixture was extracted with ether. Removal of ether at reduced pressure gave the crude triol which was immediately acetylated with acetic anhydride in pyridine. The resulting triacetate, purified by silica hplc showed $[\alpha]_D^{25} = +13.3^0$ (c = 0.9, $CHCl_3$); IR ($CHCl_3$) 2990, 1720 strong, 1450, 1390, 1240 cm^{-1} HRMS M^+-HOAc found m/z 386.2447 calc. 386.2448 ^1H NMR (360 MHz, Bz-d_6): δ 5.55 (1 H, b s), 5.18 (1 H, t, J = 7 Hz), 4.99 (1 H, d, J = 8 Hz), 4.64 (2 H, s), 4.10 (1 H, dd, J = 12, 6 Hz), 4.0 (1 H, dd, J = 12, 6 Hz), 4.0 (1 H, d, J = 15 Hz), 3.85, (1 H, d, J = 15 Hz), 3.08 (1 H, m), 2.42 (1 H, dd, J = 15, 9 Hz), 2.23 (1 H, dd, J = 15, 2 Hz), 2.13 (1 H, t, J = 7 Hz) 2.07 (1 H, m), 1.78 (1 H, dd, J = 9, 4 Hz), 1.76 (3 H, s), 1.70 (3 H, s), 1.67 (3 H, s), 1.67 (3 H, s), 1.61 (3 H, s), 1.55 (3 H, s), 1.50 (1 H, m), 0.83 (1 H, dd, J = 9, 5 Hz), 0.44 (1 H, dd, J = 5, 5 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.0 (s), 170.9 (s), 170.7 (s), 138.7 (s), 138.0 (s), 131.4 (s), 131.0 (d), 124.0 (d), 122.4 (d), 69.0 (t), 66.0 (t), 62.0 (t), 48.9 (d), 45.0 (d), 39.5 (t), 34.9 (t), 28.7 (s), 26.5 (t), 25.9 (q), 21.8 (d), 3 x 21.0 (q), 19.7 (t), 17.7 (q), 16.6 (q).

Halimedalactone (3). Halimedalactone was isolated as a minor metabolite (< 5% organic extract) from *H. tuna* and *H. scabra* collected in the Bahama Islands. The compound was obtained as a yellow oil after silica gel HPLC (45% EtOAc/isooctane). Halimedalactone showed: $[\alpha]_D^{25} = 0^o$ (c = 1.3, $CHCl_3$); unfortunately rotation at other wavelengths could not be measured due to decomposition of the compound. IR ($CHCl_3$) 2950, 1730, 1690, 1600, 1450, 1210 cm^{-1}; UV (MeOH) λ_{max} 236, ϵ = 7300. HRMS M^+ found 314.1885, (calc. for $C_{20}H_{26}O_3$ 314.1875), 133.1005 for $C_{10}H_{13}$. ^1H NMR (360 MHz, Bz-d_6): δ 9.60 (1 H, s), 6.21 (1 H, d, J = 2 Hz), 5.14 (1 H, t, J = 7 Hz), 4.50 (1 H, d, J = 8 Hz), 4.11 (1 H, dd, J = 12, 2 Hz), 3.86 (1 H, m), 2.80 (1 H, dd, J = 12, 2 Hz), 2.26 (1 H, m), 2.09 (2 H, t, J = 7 Hz), 2.00 (2 H, t, J = 7 Hz), 1.98 (1 H, m), 1.74 (1 H, m), 1.68 (3 H, s), 1.59 (3 H, s), 1.52 (3 H, s), 1.16 (1 H, m), 0.77 (1 H, dd, J = 9, 5 Hz), 0.21 (dd, J = 6, 5 Hz).

Tetraacetate (4). Tetraacetate 4 was isolated as a major metabolite in many species of *Halimeda* and often in exceptionally high concentrations (Table 1). The tetraacetate was obtained as a yellow oil after silica gel HPLC (45% EtOAc/isooctane) which showed the following spectral features: $[\alpha]_D^{25} = -1.5^o$ (c = 1.2, $CHCl_3$), IR ($CHCl_3$) 1740, 1690, 1375, 1240, 1160, 1060 cm^{-1}, UV (MeOH) λ_{max} 235, ϵ = 15,400, HRMS M^+-HOAc found 458.2302, calc. 458.2295 for $C_{28}H_{38}O_9$.

Bis-Nor Diterpenoid (5). Compound 5 was isolated in minor quantities from two species of *Halimeda* collected in Guam and from *H. scabra* collected in the Bahamas (Table 1). Compound 5 was obtained as a colorless oil, after silica gel HPLC (25% EtOAc/isooctane), which showed the following spectral features: $[\alpha]_D^{25} = +2.4^o$ (c = 0.5, $CHCl_3$); IR ($CHCl_3$) 2950 1710, 1700, 1600, 1370, 1250 cm^{-1}; UV (MeOH) λ_{max} 286 (ϵ = 3500), 249 (ϵ = 6700); HRMS M^+-HOAc found 254.1687, (calc. for $C_{20}H_{26}O_3$ 254.1665), m/z = 135.1175 for $C_{10}H_{15}$, m/z = 119.0508 for C_8H_7O. ^1H NMR (360 MHz, Bz-d_6): δ 7.58 (1 H, s), 7.42 (1 H, dd, J = 7.6, 1 Hz), 7.12 (1 H, dd, J = 7.6, 1 Hz), 6.97 (1 H, ddd, J = 7.6, 7.6, 1 Hz), 5.85 (1 H, ddd, J = 9.1, 7.0, 6.5 Hz), 5.18 (1 H, d, J = 9.1 Hz), 5.07 (1 H, t, J = 6.7 Hz), 2.83 (1 H, dd, J = 13.4, 6.5 Hz), 2.63 (1 H, dd, J = 13.4, 7.0), 2.02 (2 H, m), 1.89 (2 H, m), 1.65 (3 H, s), 1.64 (3 H, s), 1.50 (3 H, s), 1.42 (3 H, s).

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