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GERMACRENE DERIVATIVES FROM DIVERSE MARINE SOFT-CORALS (OCTOCORALLIA)

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Abstract—Examinations of the extracts of 8 taxonomically-diverse marine soft-corals (Orders Gorgonaceae, Alcyonaceae and Stolonifera, Subclass Octocorallia, Phylum Cnidaria), have resulted in the isolation of 4 new sesquiterpenoids (**1**–**4**) of the germacrene type. Three previously known germacrene derivatives (**5**–**7**), and the elemane **8**, have also been isolated, and complete ^1H and ^{13}C NMR assignments for these compounds are reported.

In the course of our investigations of various marine corals for biologically-active metabolites, we have observed that sesquiterpenoids of the germacrene class are widely distributed within the Orders Gorgonaceae, Alcyonaceae and Stolonifera (Subclass Octocorallia, Phylum Cnidaria). As a result of these studies, we wish to report here the structures of 4 new members of this sesquiterpenoid group, **1**–**4**, and to report the isolation of several known derivatives, **5**–**8**, from these same sources. Complete ^{13}C NMR assignments, aided by off-resonance decoupling, have been made for all compounds, and these appear in Table 2.

Table 1 lists the soft-corals investigated and the compounds isolated from each. Complete descriptions of the collection and isolation procedures are given in the Experimental.

The alcohol **1** was isolated, along with several other sesquiterpenoids,[1] from an extract of the Alcyonacean soft-coral *Lemnalia africana*, collected in Palau, Western Caroline Islands. Mass spectrometry established a molecular formula of $C_{15}H_{26}O$, a result reinforced by ^{13}C NMR (Table 2). The ^{13}C NMR spectrum of **1** also established that two olefinic bonds (one disubstituted and one trisubstituted) were present, and that the alcohol was totally substituted (a singlet carbon at 73.1 ppm). Consideration of the unsaturation present in the molecular formula of **1** led to the conclusion that the compound was monocyclic. The ^1H NMR spectrum, with and without the addition of $Eu(fod)_3$ shift reagent, yielded sufficient information to make the final structure assignment for this alcohol. A singlet Me group at $\delta 1.20$ indicated the tertiary alcohol was Me substituted, and an olefinic ABM pattern ($\delta 5.24$, d, $J = 15$ Hz, 5.19, dd, $J = 15, 9$ Hz) with a vicinal coupling of 15 Hz illustrated that the disubstituted olefinic bond was *trans*. Irradiation of a multiplet band at $\delta 1.39$ resulted in the collapse of two Me doublets at $\delta 0.83$ and $\delta 0.79$ ($J = 7$ Hz each), thus indicating the presence of the i-Pr group. This latter band was further coupled to a one-proton band at $\delta 2.00$ which was itself coupled to the olefinic proton at C-7 ($J = 9$ Hz). These data established the site of ring formation at C-8 and the position of the *trans* olefin at C-6–C-7. By more extensive NMR analysis (Experimental) all protons were assigned, and through a quantitative $Eu(fod)_3$ shift study, the relative stereochemistry at C-5 and C-8 was determined.

A very closely related germacrene diol, **2**, was isolated from the Pacific sea fan *Pacifigorgia media* Verrill. The very polar diol, m.p. 172–173°, analysed for $C_{15}H_{26}O_2$ by combined mass and ^{13}C NMR spectrometry, and here again two olefinic bonds analogous to those in **1** were clearly present. The ^{13}C NMR spectrum also exhibited two singlet carbons at 73.7 and 74.4 ppm, indicating that both OH groups were tertiary. In support of this latter conclusion, treatment of **2** with acetic anhydride in pyridine at room temperature failed to produce an acetylated derivative. As with **1**, the ^1H NMR features of this compound were conclusive in its final structure assignment. Three singlet Me groups at $\delta 1.08$, 1.05 and 1.30 were assigned to carbons bearing OH groups, however it was recognized that the 1.08 and 1.05 values were at somewhat high field for an i-Pr-substituted alcohol constellation. The C-6–C-7 *trans* olefin was observed at $\delta 5.37$ and 5.10 with a vicinal coupling of 16 Hz. The $\delta 5.10$ band possessed an additional coupling of 10 Hz to the methine proton at C-8, as observed in **1**. The relative stereochemistry of **2** was assigned as in **1** based upon the very close ^{13}C NMR correlation between these compounds (Table 2).

The monoacetate **3** was isolated from an extract of the previously mentioned soft-coral *Lemnalia africana*. Mass spectral and ^{13}C NMR analyses indicated a molecular formula of $C_{17}H_{28}O$, and elimination of the ester and olefinic unsaturation from this formula again showed this metabolite to be monocyclic. As in **1** and **2**, both ^{13}C and ^1H NMR data indicated that the C-5–C-8 substitution pattern remained comparable to **1** and **2**. Consideration of these data led to the proposed structure **3** for this compound, which is the simple acetate derivative of a diol recently isolated from the red seaweed *Laurencia subopposita*.[2] Treatment of **3** with a saturated solution of sodium carbonate in methanol saponified the acetate to yield the corresponding diol, which was identical, by direct comparison, to that isolated from algal sources.[2]

A novel furanogermacrene derivative, **4**, was isolated from extracts of the Caribbean sea whip *Pseudopterogorgia americana*. A combination of ^{13}C NMR and mass spectral features indicated a molecular formula of $C_{15}H_{18}O$ for this metabolite, which suggested an unusual 7 degrees of unsaturation. The utilization of the lone O atom in an α,β,β'-trisubstituted furan ring was indicated by the elimination of all other conceivable O

Table 1. Germacrene derivatives isolated from various octocorals

Order	Species	Cmpd(s)
Alcyonaceae	*Lemnalia africana* (May)	1, 3
Stolonifera	*Tubipora musica* Linnaeus	5
Gorgonaceae	*Pacifigorgia pulchra exilis* Verrill	5
	P. media Verrill	2, 5
	Pseudopterogorgia americana (Gmelin)	4
	Muricea austera Verrill	6 - 8
	M. fungifera (Valenciennes)	6 - 8

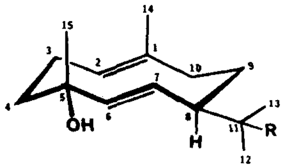
Table 2. ^{13}C NMR data for germacrene derivatives 1–8†

C#	1	2‡	3	4	5	6	7	8
1	132.5	133.7	147.6	148.4	134.4*	134.9	134.4	40.5
2	129.0	131.2	80.3	32.4*	129.0	127.3	128.9	146.0
3	23.7	24.3	31.3	25.9*	24.4	25.6	28.0	112.1
4	41.3	42.9	39.1	136.3	26.8	26.6	35.5	124.7
5	73.1	73.7*	72.1	134.2	128.8*	127.8	123.4	142.6
6	140.1	143.8	137.4	119.9	127.6	143.1	146.3	41.1
7	125.8	130.5	130.8	125.5	39.5	24.0	25.6	24.0
8	52.9	59.9	50.2	118.3	118.9	116.3	117.5	116.2
9	39.7	43.0	26.7	144.8	149.7	150.8	149.9	149.4
10	26.0	26.4	30.2	39.5	40.9	39.6	40.5	35.5
11	30.0	74.4*	32.3	123.3	121.8	121.5	121.5	119.2
12	20.6	26.6	20.6*	139.0	136.0	136.5	136.4	137.4
13	19.0	24.4	20.4*	8.8	8.8	8.2	8.8	8.1
14	16.1	17.7	114.0	113.7	16.4	16.8	16.4	18.0
15	30.8	29.6	29.6	17.1	16.2	169.5	169.6	168.7
16	-----	-----	-----	-----	-----	51.7	51.2	52.0

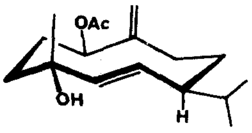
†recorded at 20MHz in $CDCl_3$ solution unless otherwise noted. Assignments are based upon off-resonance decoupling experiments yielding J_r values, multiplicities, and calculations based upon model compounds.

*signals may be reversed

‡recorded in methanol-d_4 solution



1, R = H
2, R = OH



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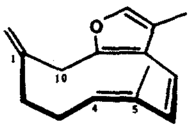

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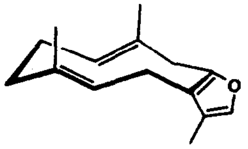

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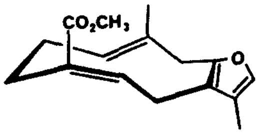

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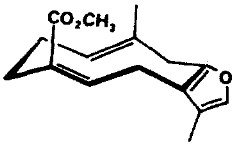

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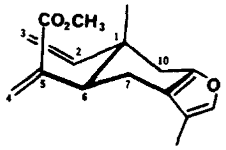

functionalities by IR and ^{13}C NMR spectral features. This classical functional group was also indicated by UV absorption at $\lambda_{max}^{MeOH} = 219$ nm, $\epsilon = 9010$ and by ^{13}C NMR features (Table 2). The ^{13}C NMR spectrum also showed that three other olefinic bonds were present, one exocyclic, one disubstituted and one trisubstituted. The final structure assignment for **4** was made based upon extensive ^1H NMR spin-decoupling experiments. The furan was recognized to bear a β-Me group by the existence of an aromatic Me at $\delta 1.88$, which was coupled to an α-furan proton observed at $\delta 7.03$. A furan Me substituent was also indicated by the high field off-resonance quartet at 8.8 ppm, in the ^{13}C NMR spectrum of this compound. In total, these data were indicative of a germacrene derivative with three unsaturations in the 10-membered ring and a β-methyl furan constructed from the i-Pr substituent. Fortuitously, several ^1H NMR features allowed a confident assignment of this compound. A highly deshielded and therefore bisallylic methylene group (C-10) was observed as a broad singlet at $\delta 3.21$. This group was allylically coupled ($J \cong 1$ Hz) to the exomethylene protons (C-14) and to no others, thus establishing this methylene between two substituted olefinic centers. The furan was initially selected as one of these olefin groups to conform to the isoprene rule. This was later confirmed by elimination of all other possibilities. The remaining unsaturation in **4** was composed of a Me-substituted olefin (a broad Me singlet at $\delta 1.50$, allylically coupled to an olefin triplet at $\delta 5.22$) and an isolated disubstituted *Z* olefin (an AB pair, $J = 11$ Hz at $\delta 5.99$ and 5.86, respectively). The trisubstituted olefin was assigned the *E* configuration based upon the shielded Me resonance (C-15) at 17.7 ppm observed in the ^{13}C NMR spectrum.[3] These features can only be accommodated in a germacrene skeleton in one way; by the construction of a diene functionality between C-4 and C-7 as shown in structure **4**. The olefinic proton at C-4 was observed, as expected, as a triplet, $J = 7.5$ Hz, coupled to an allylic methylene at $\delta 2.18$.

The data presented above strongly support the assignment of this compound as **4**, with the exception being the unexpected UV absorption at 219 nm. At first approximation, compound **4** would appear to be composed of a furan ring conjugated to a diene chromophore. Construction of molecular models with the C-4–C-5 olefin in the *E* conformation, shows that the conjugated system in **4** closely parallels the well-known tub-shaped conformation of medium-size rings such as cyclooctatetraene. The *p*-orbitals of the furan and the trisubstituted olefin are orthogonal to those of the disubstituted olefin thus eliminating conjugation between these respective chromophores. The UV absorption of this compound thus reflects only the absorption from an isolated furan.

Investigations of the Pacific gorgonians *Pacifigorgia pulchra exilis* and *P. media*, as well as the stoloniferan coral *Tubipora musica* from Palau, resulted in the isolation of furanodiene, (**5**), a compound originally isolated from the fungus *Curcuma zedoaria*.[4] This compound was identified by comparison of the literature ^1H NMR values with those from our isolate, and by its ^{13}C NMR spectrum (Table 2). Furanodiene appears to be very widely distributed in soft-corals, as it has been recently reported from two alcyonaceans of the family Xeniidae collected in north Australia.[5]

In our investigations of Pacific gorgonians of the genus Muricea, we have isolated the previously described germacrene derivatives **6–8** from *M. austera* and *M. fungifera*. Sericenine (**6**), neosericenine (**7**), and isosericenine (**8**) were originally isolated from the leaves of the composite plant *Neolitsea sericea*,[6–8] and the compounds isolated here are identical by ^1H NMR comparisons with those reported. The complete ^{13}C NMR assignments for these compounds, reported in Table 2, are in complete accord with previous structure assignments.

EXPERIMENTAL

General. IR spectra were recorded on a Perkin–Elmer Model 137 spectrophotometer and UV spectra were obtained in MeOH or hexane on a Beckmann Acta XIV instrument. Proton NMR spectra were recorded in $CDCl_3$ solution on a Varian HR-220-FT spectrometer and ^{13}C NMR spectra were obtained on a Varian CFT-20 instrument. Low resolution mass spectra were recorded at 70 eV on a Hewlett–Packard Model 5930A instrument, and high-resolution spectral measurements were obtained through the Department of Chemistry, University of California, Los Angeles. Final purifications of all metabolites were achieved by liquid chromatography (hplc) on silica gel using mixtures of ethyl acetate and isooctane.

Extractions. The frozen animal was thawed and ground with $CHCl_3$/MeOH (2/1) in a commercial blender. The mixture was warmed and then filtered, and after three repetitions the combined extracts were reduced *in vacuo*. The residue was taken up in $CHCl_3$, dried with $MgSO_4$, and after filtering, the solvent was finally removed *in vacuo* to yield the final crude extract.

Germacrene alcohol **1**. *Lemnalia africana*,[9] collection PSC-155, Palau, Western Carolina Islands, Sept. 1979, gave 41.0 gm crude extract after work-up as above. Open column chromatography of the crude extract, followed by silica gel hplc (20% EtOAc/isooctane) yielded 122 mg of **1** as a clear colorless oil. Investigation of another column fraction showed the presence of another germacrene derivative, the acetate **3**. Purification of the fraction by silica hplc (15% EtOAc/isooctane) yielded 98 mg of **3** as a colorless oil. The alcohol **1** showed $[\alpha]_D^{26} = +122°$ (*c* 1.22, $CHCl_3$) and the following spectral characteristics: IR (film) 3533, 2932, 1385, 1366, 1199, 980, 787 and 764 cm^{-1}; LRMS $M^+ m/z = 222$ for $C_{15}H_{26}O$; HRMS $M^+ - H_2O$ m/z found 204.1882, calc. 204.1872 (for $C_{15}H_{24}$); ^1H NMR (220 MHz, $CDCl_3$) $\delta 0.83$ (3H, d, $J = 7$ Hz, H-12 or H-13) 0.79 (3H, d, $J = 7$ Hz, H-13 or H-12), 1.20 (3H, s, H-15), 1.39 (3H, m, H-9 and H-11), 1.54 (3H, s, H-14), 1.59 (2H, m, H-4), 1.91 (1H, m, H-3), 2.00 (1H, m, H-8), 2.25 (2H, m, H-10) 2.49 (1H, m, $J = 10$ Hz, H-3), 4.94 (1H, broadened d, $J = 10$ Hz, H-2) 5.19 (1H, dd, $J = 15, 9$ Hz, H-7), and 5.24 (1H, d, $J = 15$ Hz, H-6).

Lanthanide shift study of germacrenoid **1**. Aliquots of $Eu(fod)_3$ in $CDCl_3$ were added to **1** in $CDCl_3$ and 220 MHz ^1H NMR spectra were recorded. Several selected ^1H bands were recorded and $\Delta\delta$ values were utilized in calculations of internuclear distances. The data in the table below were obtained following methods previously described.[10]

Diol **2**. The Pacific gorgonian *Pacifigorgia media* was collected (−5 m) at Bahia Los Frailes, Baja California Sur, Mexico in January 1979. Conventional $CHCl_3$/MeOH (2/1) extraction followed by silica column chromatography (isooctane-EtOAc mixtures) yielded mixtures containing two sesquiterpenoids. Purification of the fractions by silica hplc yielded furanodiene (**5**, 200 mg, 1.5% ext.) and the diol **2** (70 mg, 0.6% ext.). The diol was fully purified by reverse phase hplc (C_{18} − 25% H_2O in MeOH) to yield crystalline diol, m.p. 172.0–172.9°. THe diol showed the following spectral features: $[\alpha]_D - 1.3°$ (*c* 0.99, $CHCl_3$); IR (KBr) 3300, 1375, 980 cm^{-1}; HRMS $M^+ - H_2O$ $m/z = 220.1821$, calc. for $C_{15}H_{24}O$ 220.1821; LRMS M^+ $m/z = 238$, m/z 202 (100%), 187 (57%), 159 (96%), 134 (44%), 131 (42%), 119 (70%) and 105 (69%); ^1H NMR (220 MHz, MeCO-d_6) $\delta 1.05$ (3H, s), 1.08 (3H, s), 1.30 (3H, s), 1.52 (3H, s), 4.92 (1H, d, $J = 10$ Hz), 5.10 (1H, dd, $J = 16$, 10 Hz), 5.37 (1H, d, $J = 16$ Hz).

Hydroxyacetate **3**. This was isolated as 0.25% of the extract of *L. africana* (Experimental for **1**), and showed $[\alpha]_D + 23°$ (*c* 1.39, $CHCl_3$). The acetate showed identical ^1H NMR features to the synthetic acetate reported by Faulkner.[2] Hydrolysis of **3** with

Table 3.

C# Proton(s)	Induced Shift(Δδ)	r(meas)Å	r(calc)Å	% Error
2	2.21	6.2	6.31	2
6	3.20	6.0	5.84	3
7	4.91	5.1	5.06	0
8	1.84	7.1	7.06	0
14	3.11	5.8	5.80	0
15	5.90	4.6	4.58	0

sat'd Na_2CO_3 in MeOH yielded a diol (80%) which was identical, $[\alpha]_D + 90°$ (*c* 0.67, $CHCl_3$), to the natural diol earlier isolated from the red alga *Laurencia subopposita.*[2]

Furanotriene **4**. The common Caribbean sea plume *Pseudopterogorgia americana* was collected at Carrie Bow Cay, Belize, in April 1978 and stored in IPA prior to extraction. $CHCl_3$ extraction followed by silica column chromatography yielded complex mixtures of mainly unpurifiable materials. From nonpolar fractions, however, the moderately unstable furan **4** was successfully purified by silica hplc (isooctane). The furan **4** (0.3% ext) showed $[\alpha]_D$ 0° (*c* 1.5, $CHCl_3$) and the following spectral characteristics: UV(MeOH) 219 nm ($\epsilon = 9010$); IR (CCl_4 soln) 1640, 1385, 1365 cm^{-1}; LRMS M^+ $m/z = 214$ ($C_{15}H_{18}O$, 12%), m/z 199 (11%), 158 (48%), 145 (68%), 115 (50%), 105 (41%), 91 (100%), 79 (48%) and 77 (59%); ^1H NMR (220 MHz, $CDCl_3$) δ1.50 (3H, s), 1.88 (3H, s), 2.18 (broad s, 4H), 3.21 (2H, broad s), 4.54 (1H s, $J < 1$ Hz), 4.70 (1H s, $J < 1$ Hz), 5.52 (1H, t $J = 7.5$ Hz), 5.86 (1H $J = 11$ Hz, AB), 5.99 (1H, $J = 11$ Hz, AB), and 7.03 (1H, bs).

Furanodiene **5**. This was isolated, as an oil, from fractions of the extract from *P. media* (see Experimental for diol **2**). Furanodiene was also encountered as a component of the stoloniferan coral *Tubipora musica*[9] collected in Palau, Western Caroline Islands, in September 1979. Furanodiene composed 3% of the extract of this animal and showed ^1H NMR features identical with literature values.[4] Furanodiene was also isolated from extracts of the Pacific gorgonian *Pacifigorgia pulchra exilis* collected at Bahia Los Frailes, Baja California Sur, Mexico in January 1980. Furanodiene composed 5.2% of the $CHCl_3$/MeOH extract.

Sericenine (**6**), *neosericenine* (**7**) *and isosericenine* (**8**). Compounds **6**–**8** were isolated from extracts of two Pacific gorgonians, *Muricea austera* and *M. fungifera*, which were collected at Isla Socorro (May 1978) and Bahia Los Frailes (January 1980), Mexico, respectively. Both *Muricea* species contained **6** (0.6% ext.), **7** (3.2% ext.) and **8** (1.8% ext.), as isolated by silica hplc (2.5% EtOAc/isooctane). The ^{13}C NMR assignments for these compounds appear in Table 2 and their other spectral features were identical to those reported.[6–8]

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REFERENCES

[1] This same collection of *Lemnalia africana* also contained the unrelated neolemnane sesquiterpenoids which have been recently reported, see R. R. Izac, W. Fenical, B. Tagle and J. Clardy, *Tetrahedron* **37**, 2569 (1981).

[2] S. J. Wratten and D. J. Faulkner, *J. Org. Chem.* **42**, 3343 (1977).

[3] F. W. Wehrli and T. Nishida, *Fortschritte der Chemie Organischer Naturstoffe* (Edited by W. Herz, H. Grisebach and G. W. Kirby), Vol. 36, p. 24. Springer-Verlag, Wien (1979).

[4] H. Hikino, K. Agatsuma and T. Takemoto, *Tetrahedron Letters* 931 (1968).

[5] B. F. Bowden, J.-C. Braekman, J. C. Coll and S. J. Mitchell, *Aust. J. Chem.* **33**, 927 (1980).

[6] N. Hayashi, S. Hayashi and T. Matsuura, *Tetrahedron Letters* 4957 (1968).

[7] K. Takeda, I. Horibe and M. Minato, *J. Chem. Soc.* (*C*) 1547 (1970).

[8] S. Hayashi, N. Hayashi and T. Matsuura, *Tetrahedron Letters* 1999 (1968).

[9] Specimen identified by Dr. J. Verseveldt.

[10] O. J. McConnell and W. Fenical, *J. Org. Chem.* **43**, 4238 (1978).