Sequestered Chemistry of the Arminacean Nudibranch *Leminda millecra* in Algoa Bay, South Africa

Kerry L. McPhail,[†] Michael T. Davies-Coleman,*[,†] and John Starmer[‡]

Department of Chemistry, Rhodes University, Grahamstown, South Africa, and P.O. Box 3035 Koror, Palau 96940

Received February 23, 2001

Extracts of the endemic nudibranch *Leminda millecra* collected in Algoa Bay, South Africa, yielded four known metabolites, millecrones A (**1**) and B (**2**), isofuranodiene (**5**), and (+)-8-hydroxycalamenene (**9**), and nine new compounds, algoafuran (**7**), cubebenone (**8**), and a series of seven triprenylquinones and hydroquinones (**21**−**27**). A subsequent GC−MS survey of extracts of 21 of *L. millecra*'s possible octocoral prey species in Algoa Bay identified the soft coral *Alcyonium fauri* as the source of **1** and the gorgonian *Leptogorgia palma* as the species producing **2** and **8**.

The endemic South African nudibranch *Leminda millecra* Griffiths, 1985 (family Lemindidae, suborder Arminacea) is a translucent pink nudibranch with a blue-edged mantle that is expanded into well-developed parapodia. This species, lacking external gills or cerata and possessing a distinct internal morphology, is the single representative of a relatively new family of Arminacean nudibranchs.[1] *L. millecra* is reported to occur from the Cape Peninsula to Kwazulu Natal,[1] and we have observed this species to be particularly abundant at depths of 20−40 m, feeding on octocorals (mostly gorgonians), in Algoa Bay, near the coastal city of Port Elizabeth. In their earlier chemical study of *L. millecra* collected from the Transkei coast (500 km northeast of Algoa Bay), Pika and Faulkner[2] provided the first conclusive evidence of an octocoral diet for this species. Spicules characteristic of the soft corals *Alcyonium foliatum, A. valdiviae,* and *Capnella thyrsoidea* were found in the nudibranch's gut, while the skeletal structures of the sequestered metabolites, millecrones A (**1**) and B (**2**) and the millecrols A (**3**) and B (**4**), were suggestive of their octocoral origin.[2] Unfortunately, Pika and Faulkner's investigation was hampered by a paucity of nudibranch material, and they noted the presence of three minor metabolites in their *L. millecra* extracts in insufficient amounts for structure elucidation.[2] Therefore, given the abundance of *L. millecra* in Algoa Bay and our ongoing interest in identifying bioactive metabolites sequestered by Southern African nudibranchs and sea hares,[3] we present here the results of our investigation of the sequestered chemistry of *L. millecra* in Algoa Bay.

Results and Discussion

A total of 32 specimens of *L. millecra* were collected using scuba from several reefs in Algoa Bay in October 1998 and again in February 1999. The two collections of *L. millecra* were independently steeped in acetone and the acetone extracts concentrated and partitioned between EtOAc and water. The ^{1}H NMR spectra of the two EtOAc fractions were very similar, and they were consequently combined (1.78 g) and chromatographed over Si gel using a hexane/EtOAc solvent gradient. Of the seven initial chromatography fractions thus obtained, the three major fractions [1 (322 mg), 3 (440 mg), and 4 (373 mg)] were adjudged, from the plethora of methyl, oxymethine, and olefinic proton resonances in their ^{1}H NMR spectra, to be worthy of further investigation.

Additional chromatography over Si gel followed by normal-phase HPLC of the nonpolar fraction 1 yielded two known compounds: millecrone A (93 mg, 2.9 mg/animal), spectroscopically identical (^{1}H, ^{13}C, IR, and $[\alpha]_D$) to **1** isolated previously from the Transkei specimens of *L. millecra*,[2] and isofuranodiene (**5**, 11.0 mg, 0.3 mg/animal).[4] The bicyclic structure of **5** was indicated from the six degrees of unsaturation implied by the molecular formula $C_{15}H_{20}O$, established from HRFABMS data, and the presence of eight olefinic resonances in the ^{13}C NMR spectrum of this compound. Four of the latter ^{13}C resonances [δ 149.7 (s), 136.0 (d), 121.9 (s), and 118.9 (s)], together with an aromatic proton singlet (δ 7.06) in the ^{1}H NMR spectrum of **5**, confirmed the presence of a furan moiety and led us to the structure of isofuranodiene.[4] Although our ^{1}H NMR data were almost identical to those reported for isofuranodiene and furanodiene (**6**),[5] two vinyl methyl resonances (δ 16.2 and 16.5) in the ^{13}C NMR spectrum of **5** were consistent with an *E* configuration for both ring olefins as reported for isofuranodiene.[4] Interestingly, Bowden et al.[4] have proposed that the *E*,*Z* configuration of furanodiene, initially suggested three decades ago without recourse to ^{13}C NMR data,[5] is incorrect and that **5** and **6** are the same compound from a comparison of the vinyl methyl proton NMR chemical shifts of **5** with those published for **6** ($\Delta\delta \pm$ 0.02 ppm). Bicyclic, fused furanodecane metabolites appear to be confined to octocorals and three, double-bond positional isomers of **5** have been isolated from *Xenia* soft corals,[4] the Nephtheidae soft coral *Lemnalia africana*,[6] and one *Pseudopterogorgia* species.[7]

Millecrone B (**2**, 19 mg, 0.6 mg/animal) was obtained as one of the major compounds in initial chromatography fraction 3 after exhaustive normal-phase HPLC of this fraction. The spectroscopic and physical data of millecrone B were identical with those of **2** isolated during the earlier investigation of *L. millecra*.[2] In addition to **2**, fraction 3 yielded two new sesquiterpenes, algoafuran (**7**, 1.5 mg, 0.05 mg/animal) and cubebenone (**8**, 129 mg, 4 mg/animal), and the known 8-hydroxycalamenene (**9**, 16 mg, 0.5 mg/animal).

HRFABMS data revealed the molecular formula of $C_{17}H_{22}O_2$ (274.15671, Δ mmu +0.2) for algoafuran (**7**), which, in conjunction with standard acetate NMR signals (δ_C 171.1 and δ_H 2.07), suggested that **7** was a sesquiterpene monoacetate. The presence of the single acetate

* To whom correspondence should be addressed. Tel: +27 46 603 8264. Fax: +27 46 6225109. E-mail: M.Davies-Coleman@ru.ac.za.
[†] Department of Chemistry, Rhodes University.
[‡] P.O. Box 3035 Koror, Palau.

10.1021/np010085x CCC: $20.00
Published on Web 09/05/2001

carbonyl and nine olefinic carbon resonances (one overlapped) in the ^{13}C NMR spectrum of **7**, and the seven double-bond equivalents calculated from the molecular formula, implied that the structure of **7** comprised a disubstituted (δ_H, s, 7.13 and 6.16) furan ring substituted with a methyl group and a linear, unsaturated side chain. The ^1H NMR data of algoafuran differed from similar sesquiterpenes, e.g., **10**, isolated from the Mediterranean soft coral *Alcyonium palmatum*.[8] The replacement of the vinyl methyl singlet (δ 1.62) in **10** with an oxymethylene singlet (δ 4.98) in the ^1H NMR spectrum of **7** placed the acetate group at C-10′ in this compound. This structural assignment was supported by 2D NMR data including three-bond HMBC correlations from the methylene protons (H_2-10′) to the acetate carbonyl (δ 171.1), C-1′ (δ 118.0), C-2′ (δ 134.6), and C-3′ (δ 35.6). The ^1H and ^{13}C NMR chemical shifts for both the 2,4-disubstituted furan ring and the terminal diene portion of the unsaturated side chain of compound **7** were consistent with the analogous chemical shifts in **10**. The *E*/*Z* stereochemistry of the Δ^1 olefin was not determined. Several similar 2,4-disubstituted furanosesquiterpenes with C-2 decatriene side chains have been reported from species of *Sinularia* soft corals.[9]

A molecular formula of $C_{15}H_{22}O$ (*m*/*z* 219.1748, M + 1, Δmmu −0.1) for **8** was established from HRFABMS data. A strongly deshielded carbonyl resonance (δ 209.0) in the ^{13}C NMR spectrum of **8**, together with two olefinic carbon resonances [δ 123.4 (d) and 177.9 (s)], immediately suggested the presence of an α,β-unsaturated carbonyl moiety. The ^{13}C NMR spectrum of **11**, prepared via catalytic hydrogenation of **8**, revealed no vinylic carbon signals and instead contained additional methylene (δ 40.9) and methine carbon resonances (δ 29.6). As expected, the IR absorptions attributed to the α,β-unsaturated ketone functionality in **8** (1694, 1607 cm^{-1}) were shifted to 1721 cm^{-1} in the IR spectrum of the saturated ketone.

A tricyclic structure for **8** was indicated from the remaining three (of five) double-bond equivalents implied by the molecular formula. A cubebane structure was the only tricyclic system compatible with the spectral data. The isopropyl and the methyl substituents on the cubebane skeleton were readily identified from the ^1H and COSY NMR data [a methine proton octet coupled to two closely overlapped methyl doublets (δ 0.84, 0.88, J = 7 Hz) and a methine proton septet coupled to a methyl doublet (δ 0.89, J = 6 Hz)]. The latter methyl doublet showed two- and three-bond HMBC correlations to C-1 (δ 26.3), C-2 (δ 30.5), and C-9 (δ 42.8), thus placing the methyl substituent at C-1. Further HMBC correlations from the isopropyl methine proton to C-4 (δ 45.2) and C-3/C-10 (δ 26.3) positioned the isopropyl group at C-4. The remaining deshielded methyl substituent (δ 2.10, H_3-15) was unequivocally placed on the only olefinic quaternary carbon C-6 (δ 177.9) from a two-bond HMBC correlation from the methyl protons to C-6 and three-bond HMBC correlations to C-5 (δ 35.5) and C-7 (δ 123.4). Hence, the absence of a methyl substituent on the only sp^3 quaternary carbon (δ 42.8) dictated the placement of the quaternary carbon at the junction of the three rings (C-9) in **8**. The connectivity of the three rings was clearly delineated by a plethora of two- and three-bond HMBC correlations from methine proton H-10 (δ 1.31) and vinylic proton H-7 (δ 5.31) to several neighboring carbon atoms as shown in Figure 1.

NOESY correlations between the more shielded of the two H-2 resonances in **8** (δ 0.64, H-2β) and H-4 and H-5 placed these three protons in axial positions on the same side of the molecule and implied that the C-4 isopropyl

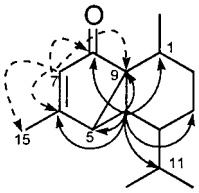


Figure 1. Selected HMBC correlations from H-7 (dashed lines) and H-10 (solid lines) for compound **8**.

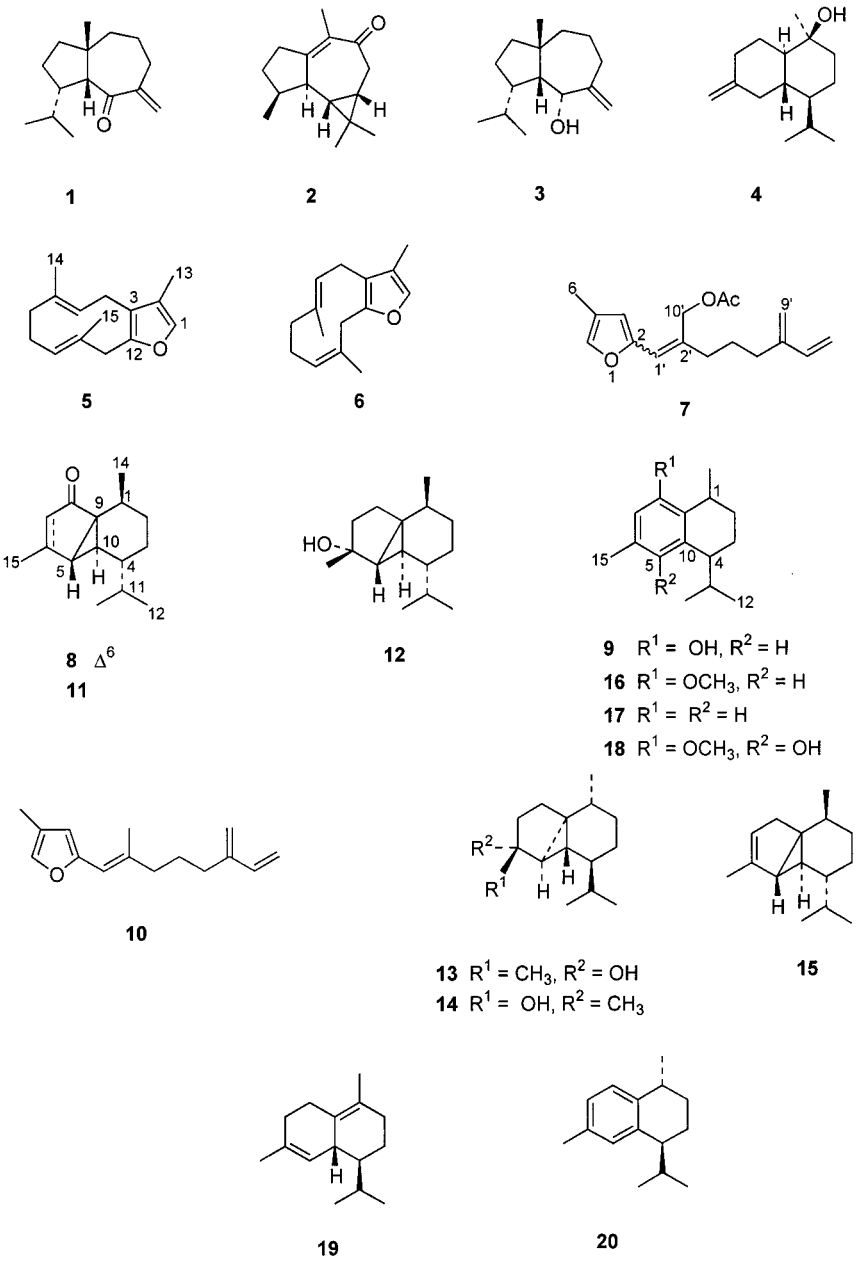
substituent was equatorial. The absence of a NOESY correlation between H-2β and H-1 together with the coupling pattern of H-2β (br q, J = 12 Hz) were suggestive of a trans relationship between H-2β and H-1 and thus required an equatorial methyl substituent at C-1. A molecular model indicated that $J_{4,10} = J_{5,10} = 3$ Hz was appropriate for an equatorial orientation for H-10 relative to the axial H-4 and H-5 protons, thus establishing the stereochemistry at the C-10 ring junction. The structure of cubebenone (**8**, $[\alpha]_D$ + 126) is similar to that of (+)-cubebol (**12**, $[\alpha]_D$ + 60.2) isolated from an Australian *Cespitularia* soft coral,[10] the C-6 epimer (**13**, $[\alpha]_D$ −42) of the terrestrial plant metabolite, (−)-cubebol (**14**, $[\alpha]_D$ −61.6),[11] reported from the brown alga *Dictyopteris divarcia*,[12] and (+)-α-cubebene (**15**, $[\alpha]_D$ +23.6) from the gorgonian *Psuedoplexaura porosa*.[13] Interestingly, Weinheimer et al.[13] observed that each of the sesquiterpenes isolated in their study of 10 gorgonian species from the Caribbean was enantiomeric with the form commonly found in plants. This observation, coupled with the positive optical rotation of **8** and our subsequent discovery of **8** in *Leptogorgia palma* (see later), influenced our assignment of the stereochemistry of cubebenone.

Compound **9** coeluted with millecrone B during normal-phase HPLC of initial chromatography fraction 3 (19:1 hexane/EtOAc). After an unsuccessful attempt to separate the two compounds using normal-phase HPLC (hexane) we serendipitously discovered that further normal-phase HPLC ($CHCl_3$) of the mixture eluted only **9**, enabling us to subsequently obtain **2** by washing the column with EtOAc. A molecular formula of $C_{15}H_{20}O$ for **9** was confirmed from HREIMS data (218.1679, Δmmu + 0.8), while a broad hydroxyl absorption band centered at 3437 cm^{-1} in the IR spectrum of **9** established the presence of an alcohol functionality in this compound. A comparison of the ^{13}C NMR data of **9** with those of 8-methoxycalamenene (**16**), isolated from the Red Sea gorgonian *Subergorgia hicksoni*,[14] revealed the absence of a methoxy methyl group in **9** and only slight differences (2−5 ppm) in the shifts of C-7, C-8, and C-9 (Table 1). Unfortunately, overlap of the proton signals of H-2 (δ 1.97) and H-11 (δ 1.99), and the two H-3 protons (δ 1.80), prevented unambiguous assignment of the relative stereochemistry of the two chiral centers in **9**. 8-Hydroxycalamenene has been previously isolated from the liverwort *Bazzania trilobata*[15] and synthesized as a racemate.[16] Marine octocoral secondary metabolites related to **9** and **16** include (+)-calamenene (**17**) from the gorgonian *Pseudoplexaura porosa*[13] and 5-hydroxy-8-methoxycalamenene (**18**) from *S. hicksoni*.[14] More recently, 11 calamenenes, many highly oxygenated, were isolated from the Australian soft coral *Lemnalia cervicornis*.[17] Interestingly, Suzuki et al.[12] were able to convert **13** into a mixture of (−)-α-cubebene, (−)-β cubebene, (+)-δ-cadinene (**19**), and (−)-*trans*-calamenene (**20**) by heating (5 h, ca. 130 °C). Conversion of **13** to a mixture of **19** and other hydrocarbons was also achieved on prolonged contact of **13** with Si gel. Therefore, it occurred to us that if a similar thermal, or silica gel induced, conversion of **8** into **9** could be achieved,

Table 1. ^{13}C (100 MHz, $CDCl_3$) and ^{1}H (400 MHz, $CDCl_3$) NMR Data for Compounds **8** and **9** and ^{13}C NMR (22.63 MHz, $CDCl_3$) Data for Compound **16**[14]

	8		**9**		**16**
atom no.	δ_C ppm (mult.)	δ_H ppm (mult., *J*/Hz)	δ_C ppm (mult.)	δ_H ppm (mult., *J*/Hz)	δ_C ppm (mult.)
1	26.3 (d)	2.44 (sept, 6)	26.6 (d)	3.06 (qn, 6)	26.6 (d)
2	30.5 (t)	0.64 (br q, 12) 1.74 (m)	27.2 (t)	1.97 (m)	27.2 (t)
3	26.3 (t)	0.88 (qd, 2, 14) 1.41 (m)	19.1 (t)	1.80 (br m)	19.2 (t)
4	45.2 (d)	1.07 (m)	43.1 (d)	2.45 (sext, 3)	43.1 (d)
5	35.5 (d)	1.89 (d, 3)	123.0 (d)	6.58 (s)	122.6 (d)
6	177.9 (s)		135.0 (s)		134.6 (s)
7	123.4 (d)	5.31 (s)	113.3 (d)	6.42 (s)	108.6 (d)
8	209.0 (s)		153.0 (s)		157.2 (s)
9	42.8 (s)		126.0 (s)		128.6 (s)
10	54.2 (d)	1.31 (t, 3)	141.2 (s)		140.7 (s)
11	32.8 (d)	1.54 (octet, 7)	33.2 (d)	1.99 (m)	33.3 (d)
12	19.6 (q)	0.88 (d, 7)	19.6 (q)	0.82 (d, 7)	19.2 (q)
13	19.6 (q)	0.84 (d, 7)	22.1 (q)	0.97 (d, 7)	19.6 (q)
14	19.7 (q)	0.89 (d, 6)	21.2 (q)	1.19 (d, 7)	22.1 (q)
15	18.8 (q)	2.10 (s)	21.1 (q)	2.24 (s)	21.5 (q)
OCH_3					55.6 (q)

Chart 1



the latter compound might be considered as an artifact derived from **8** during the workup and subsequent chromatography of the *L. millecra* extracts. However, no **9** was formed from **8** (as adjudged by periodic GC analysis) either on heating (6 h, 135−150 °C) or when **8** was left to either stand at room temperature for prolonged periods in $CHCl_3$

Table 2. ^{13}C NMR ($CDCl_3$, 100 MHz) Data for Compounds **21**−**27**[a]

carbon	21	22	23	24	25	26	27
1	187.8 (s)	187.8 (s)	147.1 (s)	148.8(s)	141.1 (s)	146.7 (s)	146.9 (s)
2	148.4 (s)	148.5 (s)	125.3 (s)	124.8 (s)	130.4 (s)	119.5 (d)	125.3 (s)
3	132.3 (d)	132.4 (d)	115.8 (d)	115.4 (d)	115.1 (d)	112.5 (d)	115.4 (d)
4	188.3 (s)	188.4 (s)	148.0 (s)	146.4 (s)	153.3 (s)	147.5 (s)	148.2 (s)
5	145.6 (s)	145.6 (s)	122.4 (s)	122.3 (s)	122.7 (s)	124.6 (s)	122.7 (s)
6	133.5 (d)	133.5 (d)	117.8 (d)	117.7 (d)	123.5 (d)	118.1 (d)	118.2 (d)
7	15.4 (q)	15.4 (q)	15.5 (q)	15.5 (q)	15.6 (q)	15.9 (q)	15.5 (q)
1′	27.1 (t)	27.1 (t)	28.3 (t)	27.5 (t)	27.0 (t)	122.4 (d)	28.0 (t)
2′	118.3 (d)	118.0 (d)	122.2 (d)	122.4(d)	122.3 (d)	129.5 (d)	123.1 (d)
3′	139.5 (s)	139.8 (s)	136.8 (s)	137.3 (s)	137.4 (s)	77.9 (s)	136.7 (s)
4′	39.2 (t)	39.7 (t)	39.0 (t)	39.8 (t)	39.9 (t)	40.6 (t)	39.1 (t)
5′	26.5 (t)	26.5 (t)	25.8 (t)	25.4 (t)	25.1 (t)	22.9 (t)	25.3 (t)
6′	128.9 (d)	33.5 (t)	128.8 (d)	33.1 (t)	33.0 (t)	129.4 (d)	128.4 (d)
7′	129.4 (s)	158.6 (s)	128.9 (s)	161.0 (s)	161.7 (s)	129.2 (s)	132.4 (s)
8′	54.3 (t)	124.3 (d)	53.7 (t)	123.9 (d)	123.8 (d)	54.4 (t)	48.2 (t)
9′	209.3 (s)	200.6 (s)	211.1 (s)	202.8 (s)	203.6 (s)	209.6 (s)	66.5 (d)
10′	50.7 (t)	53.5 (t)	51.1 (t)	53.7 (t)	53.8 (t)	50.6 (t)	46.0 (t)
11′	24.4 (d)	25.1 (d)	24.6 (d)	25.7 (d)	25.9 (d)	24.4 (d)	24.7 (d)
12′	22.5 (q)	22.7 (q)	22.5 (q)	22.6 (q)	22.6 (q)	22.5 (q)	23.3 (q)
13′	22.5 (q)	22.7 (q)	22.5 (q)	22.6 (q)	22.6 (q)	22.5 (q)	22.3 (q)
14′	16.4 (q)	25.5 (q)	17.0 (q)	25.2 (q)	25.1 (q)	16.3 (q)	16.2 (q)
15′	16.0 (q)	16.1 (q)	15.9 (q)	15.8 (q)	15.7 (q)	26.1 (q)	15.6 (q)
Ac CO					170.0 (s)		
Ac Me					20.8 (q)		

[a] Multiplicities inferred from DEPT 135 experiments.

or in contact with Si gel. In addition **8** was found to co-occur consistently with millecrone B, and not **9**, in the extracts we obtained from individual nudibranchs and the gorgonian *Leptogorgia palma* in the GC-screening program described later.

The remaining seven new compounds (**21**−**27**), isolated by further HPLC of the initial chromatography fractions 3 and 4, are all triprenyltoluquinones and toluhydroquinones related to the triprenylhydroquinone, rietone (**28**), which was previously obtained from the South African soft coral *Alcyonium fauri*.[18] Compounds **21**−**27**, however, differ from rietone in that they possess a methyl, not a methylene carboxy, substituent at C-5 in the quinone/hydroquinone ring, and they do not have an acetoxy or hydroxy substituent α to the side-chain carbonyl group. In this respect compounds **21**−**27** are more similar to the glycoside toluhydroquinone, moritoside (**29**), isolated from a Japanese gorgonian, *Euplexaura* sp.[19]

The least polar, bright yellow compounds **21** (36 mg, 1.1 mg/animal) and **22** (15 mg, 0.5 mg/animal), obtained from initial chromatography fractions 3 and 4, displayed UV (250 and 252 nm, respectively) and IR (1657 and 1656 cm^{-1}, respectively) absorptions characteristic of quinones.[20] HREIMS data established a molecular formula $C_{22}H_{30}O_3$ (342.2198, Δ mmu +0.5) for **21**, while its ^{13}C NMR spectrum showed 22 carbon resonances (Table 2), of which eight were vinylic, two were typical of quinone carbonyls (δ 187.8 and 188.3),[20] and one was a ketone resonance (δ 209.3). With seven of the eight double-bond equivalents suggested by the molecular formula thus accounted for, we propose a disubstituted quinone structure for **21**. The 3,7,11-trimethyl-2,6-dodecadien-9-one side chain of **21** was easily delineated by two- and three-bond HMBC correlations from the ^1H NMR signals of each of the regularly spaced methyl groups to the ^{13}C resonances of their three nearest neighboring carbon atoms (Figure 2). The substitution pattern around the quinone ring could be similarly established from the HMBC data (Figure 2). The ^{13}C chemical shifts of the olefinic methyl groups H_3-14′ (δ 16.4) and H_3-15′ (δ16.0) implied an *E* geometry for the two double bonds ($\Delta^{2'}$ and $\Delta^{6'}$) in the side chain.[18,21]

The structure of the isomeric toluquinone **22** ($C_{22}H_{30}O_3$, HRFABMS, 343.2273, M + 1, Δmmu −0.02) was readily resolved by comparison of the ^{13}C NMR data of this compound with those of **21** (Table 2). A relatively shielded ^{13}C resonance for the side-chain carbonyl carbon (C-9′, δ 200.6) in **22** (cf. δ 209.3 for **21**) and significant differences in the chemical shifts of C-6′, C-7′, C-8′, and C-14′ (Table 2) suggested a double-bond shift from $\Delta^{6'}$ in **21** to $\Delta^{7'}$ in **22**. Further evidence for the presence of an α,β-unsaturated carbonyl in **22** was provided by a strong IR absorption at 1682 cm^{-1} (cf. 1712 cm^{-1} for **21**) and a deshielded vinylic proton singlet (δ 6.08) in the ^1H NMR spectrum (cf. δ 5.21 for **21**). The ^{13}C chemical shifts of the two allylic methyls H_3-15′ (δ 16.1) and H_3-14′ (δ 25.5) established the *E* and *Z* configurations of the ($\Delta^{2'}$ and $\Delta^{7'}$) olefins, respectively.[21,22] Additional support for the latter stereochemical assignment was provided by a prominent NOESY correlation observed between H-8′ and H_3-14′. Similar arguments were used to determine the side-chain double-bond stereochemistry in compounds **23**−**27**.

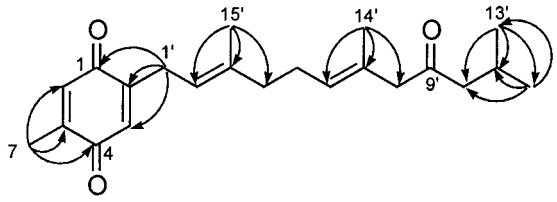
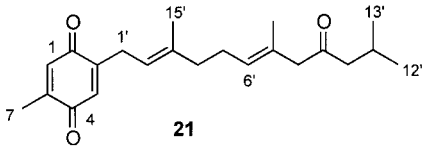


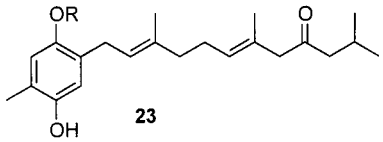
Figure 2. Selected HMBC correlations for compund **21**.

In addition to the toluquinones **21** and **22**, their toluhydroquinone analogues, **23** (154.7 mg, 4.8 mg/animal) and **24** (9.8 mg, 0.3 mg/animal), were isolated from initial chromatography fraction 4. HRFABMS established a molecular formula of $C_{22}H_{32}O_3$ for the latter isomeric compounds (344.23514, Δmmu −0.01), which both gave hydroxyl (3396 and 3369 cm^{-1}, respectively) IR absorptions. No quinone carbonyl ^{13}C resonances were evident in the ^{13}C NMR spectra of **23** and **24**, and an inspection of the ^{13}C NMR spectral data of these two metabolites (Table 2) revealed significant differences only in the chemical shifts of the six ring carbons, compatible with the trend expected for a hydroquinone as opposed to a quinone ring structure.[23] Furthermore, inspection of the ^1H NMR spectrum of **23** revealed the presence of two broad D_2O-exchangeable proton signals (δ 4.83 and 5.72) attributed to the two phenolic protons, while HMBC data was again instrumental in establishing the substitution pattern around the aromatic ring. The structural elucidation of **24** followed from an analogous comparison of the spectral data of this compound with those of **22**.

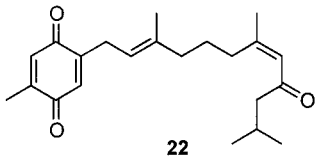
Chart 2



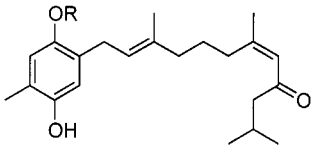
21



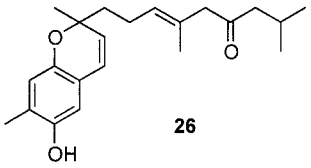
23



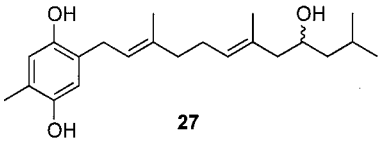
22



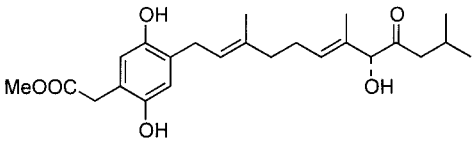
24 R = H
25 R = Ac



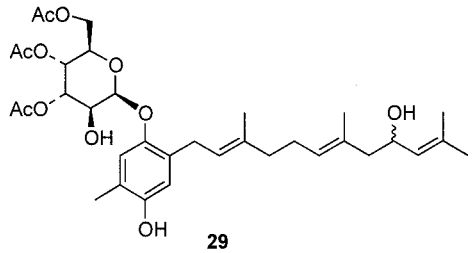
26



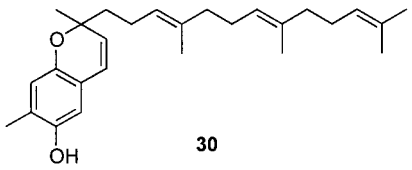
27



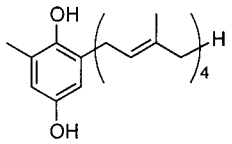
28



29



30



31

Further HPLC of initial chromatography fraction 3 also yielded **25** (3.1 mg, 0.1 mg/animal) and **26** (8.2 mg, 0.3 mg/animal). A molecular formula of $C_{24}H_{34}O_4$ (386.24567, Δmmu −0.04) for **25** was established by HRFABMS and revealed a molecular mass discrepancy of 58 amu between **24** and **25**. The methyl singlet at δ 2.27 in the ^{1}H NMR spectrum of **25**, linked through an HMBC correlation to a ^{13}C resonance at δ 170.0, together with a strong absorbance at 1760 cm^{-1} in the IR spectrum of **25** implied that the mass difference between **25** and **24** was attributable to the presence of a single acetate moiety in **25**. A comparison of the ^{13}C NMR data for **25** with those of **24** (Table 2) indicated that the structure of the 3,7,11-trimethyl-2,7-dodecadien-9-one side chain in both of these compounds was identical, thus requiring the acetate moeity to reside at either C-1 or C-4 in the toluhydroquinone ring. Accordingly, a two-bond HMBC correlation from the phenolic proton signal (δ 8.13) in the ^{1}H NMR spectrum of **25** to C-4 (δ 122.7) placed the hydroxyl group on this carbon atom and the acetate moiety at C-1.

HRFABMS established a molecular formula of $C_{22}H_{30}O_3$ (342.21940, Δmmu −0.1) for **26**, whose ^{13}C NMR data were consistent with those of **21** and **23** except for marked differences between the chemical shifts of side-chain carbons C-1′−C-3′ and C-15′ in these compounds (Table 2). The 10 olefinic carbons and one carbonyl moiety, apparent in the ^{13}C NMR spectrum of **26**, accounted for only six of the eight degrees of unsaturation inferred from the molecular formula and required **26** to be bicyclic. A chromenol structural motif, arising from cyclization of the C-1′−C-3′ fragment of the 3,7,11-trimethyl-2,6-dodecadien-9-one side-chain, was deemed a logical bicyclic structure for **26** given the discrepancies in ^{13}C chemical shifts between **21** and **23** and **26** alluded to above. The chromenol structure of **26** was unequivocally confirmed by comparison of the ^{1}H and^{13}C NMR chemical shifts for the proposed bicyclic fragment of **26** with those reported for an analogous chromenol moiety in the tetraprenyltoluhydroquinone (**30**) isolated from an unidentified Australian soft coral of the genus *Nephthea*.[20]The ^{13}C NMR spectrum of **27** (3.6 mg, 0.1 mg/animal) differed from those of the other toluhydroquinones (**23**−**25**) in that it lacked a ketone carbonyl resonance. HRFABMS (346.25074, Δmmu −0.1) established a molecular formula of $C_{22}H_{34}O_3$ for **27**, which together with a D_2O-exchangeable signal (δ 1.25) in the ^{1}H NMR spectrum and a hydroxy methine carbon resonance (δ 66.5) in the ^{13}C NMR spectrum of this compound (Table 2) was suggestive of a reduced side-chain carbonyl moiety. Placement of the secondary alcohol functionality at C-9′ was confirmed by strong COSY couplings from the oxymethine proton (δ 3.76) to the vicinal diastereotopic methylene protons H_2-8′ (δ 1.96 and 2.21) and H_2-10′ (δ

1.22 and 1.49). Unfortunately, a paucity of material prevented the assignment of the C-9′ absolute stereochemistry employing Mosher's standard MTPA ester procedure.

Linear, polyprenyl quinones and hydroquinones are not common in octocorals. With the exception of the triprenylated rietone from *Alcyonium fauri*,[18] only tetraprenylhydroquinones and quinones have been isolated thus far from soft corals, in particular from species of the genera *Sinularia*[23,24]and *Nepthea*.[20,23] Two investigations of *Euplexaura* gorgonians have yielded six triprenyltoluhydroquinones bearing a glycoside substituent at C-1, e.g., **29**.[19,25] While all of the prenylated quinones and hydroquinones reported from octocorals have a methyl-derived substituent at C-5, as is evident in the *Leminda* compounds described here, analogous polyprenylated quinone and hydroquinone metabolites from brown algae have the methyl substituent at C-6, e.g., compound **31**.[26] Therefore, although prenylated quinones and hydroquinones are ubiqitous in brown algae,[27] and relatively rare in octocorals, we speculate that the 2,5-alkylated toluquinones and toluhydroquinones isolated from *L. millecra* are true octocoral metabolites and are not sequestered by the nudibranch from algae.

The metabolites reported in this paper were obtained by combining many individual specimens of *L. millecra* collected from different dive sites in Algoa Bay at various times. While combining and extracting relatively large numbers of nudibranchs is a convenient way to obtain an overview of the range of natural products sequestered by a nudibranch species inhabiting a well-defined subtidal region, e.g., Algoa Bay, this procedure provides no information about the dietary selectivity of individual nudibranchs. Given the paucity of extract provided by single specimens of *L. millecra*, and the perceived volatility of the majority of the identified *L. millecra* sesquiterpene metabolites, we anticipated that GC would be a suitable technique to analyze individual nudibranch extracts. While compounds **1**, **2**, **8**, **9**, and **26** were readily amenable to GC separation on a DB-1 GC column, we were unable to establish any GC conditions that could separate the remaining quinones and hydroquinones (**21**−**25**, **27**). Frustratingly, the furanosesquiterpenes **5** and **7** proved to be notoriously unstable and had degraded prior to us establishing suitable GC conditions for their detection.

Although we were unable to identify all of the *L. millecra* metabolites we had isolated using GC, we were confident that this technique would be useful for determining the dietary preferences of individual specimens of *L. millecra* in Algoa Bay. Accordingly, eight *L. millecra* specimens were collected using scuba from "White Sands" in Algoa Bay (March 2000), together with three different small gorgonians upon which three of the nudibranchs were found. The nudibranchs were individually extracted in acetone and each of the eight extracts subjected to GC analysis. The GC analyses revealed that each of the eight nudibranch acetone extracts contained **8** (t_R = 21.50 min) as a major constituent with a small, although significant, amount of **2** (t_R = 25.25 min) present. Unequivocal confirmation of the identity of both of these GC peaks was provided by subsequent GC−MS analysis.

The three small gorgonians collected with the nudibranchs were also individually extracted, and of these three extracts only one, from *Leptogorgia palma*, matched the GC profile (t_R = 21.50 and 25.25 min) of the "White Sands" *L. millecra* specimens. These data would suggest that *L. palma* is an important source of the sequestered metabolites of *L. millecra* in Algoa Bay. GC−MS analysis of crude extracts of 18 octocorals (mostly soft corals) collected from Algoa Bay during large-scale collections of marine invertebrates by the Coral Reef Research Foundation revealed the presence of **1** in two of the extracts: OCDN 6174 (an unidentified *Alcyonium* species) and OCDN 6176 (*Alcyonium fauri*). No evidence of compounds **2**, **8**, **9**, and **26** was found in any of the 18 NCI extracts.

Experimental Section

General Experimental Procedures. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker AVANCE 400 NMR spectrometer. Chemical shifts are reported in ppm and referenced to residual undeuterated solvent resonances. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Infrared data were obtained on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer with compounds as films (neat) on NaCl disks. LRMS were recorded on a Finnigan GCQ spectrometer at 70 eV. HREIMS (Micromass Autospec-TOF spectrometer) and HRFABMS (Micromass 70-70E spectrometer) were obtained by Dr. P. Boshoff and Prof. L. Fourie of the Mass Spectrometry Units at the Cape Technikon, Cape Town, and the University of Potchefstroom, Potchefstroom, respectively. Normal-phase and reverse-phase semipreparative HPLC separations were performed on Whatman Magnum 9 Partisil 10 column and Phenomenex Luna 10 μ C18 columns, respectively. GC analyses were performed on a Hewlett-Packard 6890C FID gas chromatograph using a DB-1 capillary column (0.25 mm × 30 m) and front inlet and detector temperatures of 250 °C. The split ratio was 15:1, and the injection volume 1 μL. The oven temperature profile used for the analysis of all pure compounds and crude extracts was as follows: 5 min hold at 40 °C, 10 °C/min increment to 150 °C, 5 min hold at 150 °C, 5 °C/min increment to 280 °C, 10 min hold at 280 °C.

Invertebrate Material. Nineteen specimens of the common, and easily identifiable, nudibranch *Leminda millecra* were collected in October 1998 from Algoa Bay by hand using scuba equipment (−20 to 40 m). A further 13 specimens were similarly collected from Algoa Bay in February 1999. In March 2000, eight *L. millecra* specimens, and three species of gorgonians (*Leptogorgia* sp., *L. palma*, and an *Acabaria* species) upon which they were found, were collected from two sites (−18m and −23 m) at White Sands in Algoa Bay for GC analysis. A voucher specimen of *L. millecra* is retained in the Rhodes University marine invertebrate collection (KUPE98.012).

Extraction and Isolation of *L. millecra* Metabolites. The acetone extracts of the nudibranch specimens from the 1998 and 1999 collections were worked up separately and initially partitioned between EtOAc and water. The two concentrated EtOAc partition fractions thus obtained gave identical ^{1}H NMR spectra and therefore were combined (1.78 g) and subjected to initial chromatography on a Si gel column using gradient elution (hexane, hexane/EtOAc, 8:2, 1:1, and EtOAc). Fractions collected were combined according to their TLC profiles to give seven main fractions, of which fractions 1 (322 mg), 3 (440 mg), and 4 (373 mg) were selected for further purification. Normal-phase HPLC of initial chromatography fraction 1 (39:1 and 79:1 hexane−EtOAc) afforded **1** (93 mg, 2.9 mg/animal) and **5** (11.0 mg, 0.3 mg/animal). Additional Si gel chromatography (hexane−EtOAc) of initial chromatography fraction 3 followed by normal-phase HPLC with various eluents (9:1, 17:3, 19:1, 37:3 hexane−EtOAc, and $CHCl_3$/EtOAc) of selected fractions afforded **2** (19 mg, 0.6 mg/animal), **7** (1.5 mg, 0.05 mg/animal), **8** (129 mg, 4.0 mg/animal), **9** (16 mg, 0.5 mg/animal), **21** (9.2 mg), **22** (8.2 mg), **25** (3.1 mg, 0.1 mg/animal), and **26** (8.3 mg, 0.5 mg/animal). The application of a similar HPLC separation protocol to initial chromatography fraction 4 afforded more of compounds **21** (19.3 mg, 0.9 mg/animal in total) and **22** (6.4 mg, 0.5 mg/animal in total), in addition to **24** (154.7 mg, 4.8 mg/animal), **25** (9.8 mg, 0.3 mg/animal), **26** (8.3 mg, 0.3 mg/animal), and **27** (3.6 mg, 0.1 mg/animal).

Millecrone A (1): colorless oil; $[\alpha]^{21}_D$ +41° (*c* 0.60, $CHCl_3$), lit.[2] $[\alpha]_D$ +39.5° (*c* 1.36, $CHCl_3$); IR, ^{1}H, and ^{13}C NMR data in

agreement with published values;[2] HRFABMS *m/z* 221.1905 (calcd for $C_{15}H_{25}O$, 221.1905).

Millecrone B (2): colorless oil; $[\alpha]^{20}_D$ +155° (*c* 0.65, $CHCl_3$); lit.[2] $[\alpha]_D$ +151.0° (*c* 0.32, heptane); IR, ^1H, and ^{13}C NMR data in agreement with published values;[2] HREIMS *m/z* 218.1677 (calcd for $C_{15}H_{22}O$, 218.1669).

Isofuranodiene (5): amorphous solid; IR, ^1H, and ^{13}C NMR data in agreement with published values;[4] HRFABMS *m/z* 216.1514 (calcd for $C_{15}H_{20}O$, 216.1514).

Algoafuran (7): colorless oil; IR ν_{max} 2930, 1739, 1371, 1234, 1023, 894 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.71 (2H, m, H_2-4′), 2.00 (3H, s, H_3-6), 2.07 (3H, s, H_3-12′), 2.24 (4H, t, J = 8 Hz, H_2-3′, H_2-5′), 4.98 (2H, s, H_2-10′), 5.01 (2H, d, J = 18 Hz, H_2-9′), 5.04 (H, d, J = 17 Hz, H-8′), 5.22 (1H, d, J = 18 Hz, H-8′), 6.10 (1H, s, H-3), 6.16 (1H, s, H-1′), 6.36 (1H, dd, J = 18 Hz, H-7′), 7.13 (1H, s, H-5) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 9.6 (q, C-6), 21.0 (q, C−Ac−Me), 26.7 (t, C-4′), 31.0 (t, C-5′), 35.6 (t, C-3′), 63.3 (t, C-10′), 112.5 (d, C-3), 113.3 (t, C-8′), 115.9 (t, C-9′), 118.0 (d, C-1′), 121.5 (s, C-4), 134.6 (s, C-2′), 138.8 (d, C-5), 138.8 (d, C-7′), 146.0 (s, C-6′), 151.8 (s, C-2), 171.1 (s, Ac−CO) ppm; HRFABMS *m/z* 274.1567 (calcd for $C_{17}H_{22}O_3$, 274.1569).

Cubebenone (8): yellow oil; $[\alpha]^{23}_D$ +126° (*c* 0.67, $CHCl_3$); IR ν_{max} 1694, 1607, 1447, 1377, 1323, 1247, 1028, 872, 831, 610 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* (rel int) 218 (26), 203 (33), 175 (31), 161 (25), 147 (32), 136 (100), 121 (58), 105 (35), 91 (32); HRFABMS *m/z* 219.1748 (calcd for $C_{15}H_{23}O$, 219.1749).

8-Hydroxycalamenene (9): yellow oil; $[\alpha]^{22}_D$ +36° (*c* 0.53, $CHCl_3$); IR ν_{max} 3437, 1619, 1579, 1464, 1287, 1240, 1165, 1030, 974, 904, 842 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* (rel int) 218 (79), 176 (73), 175 (100), 160 (33), 159 (28), 147 (34), 121 (20); HREIMS *m/z* 218.1679 (calcd for $C_{15}H_{22}O$, 218.1671).

5-Methyl-2-[(2′*E*,6′*E*)-3′,7′,11′-trimethyl-2′,6′-dodecadien-9′-onyl]benzo-1,4-quinone (21): bright yellow oil; λ_{max} (MeOH) nm (log ϵ) 252 (3.76); IR ν_{max} 1712, 1656, 1614, 1445, 1366, 1284, 1238, 1133, 909 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.89 (6H, d, J = 7 Hz, H_3-12′, H_3-13′), 1.60 (3H, s, H_3-14′), 1.61 (3H, s, H_3-15′), 2.02 (3H, s, H_3-7), 2.09 (1H, t, J = 7 Hz, H-11′), 2.07 (2H, m, H_2-4′), 2.16 (2H, m, H_2-5′), 2.28 (2H, d, J = 7 Hz, H_2-10′), 3.02 (2H, s, H_2-8′), 3.10 (2H, d, J = 7 Hz, H_2-1′), 5.15 (1H, t, J = 7 Hz, H-2′), 5.21 (1H, t, J = 7 Hz, H-6′), 6.48 (1H, s, H-3), 6.58 (1H, d, J = 1 Hz, H-6) ppm; ^{13}C NMR data, see Table 2; EIMS *m/z* (rel int) 342 (8), 215 (27), 190 (36), 189 (21), 175 (69), 151 (18), 137 (20), 121 (22), 85 (50), 57 (100), 41 (33); HREIMS *m/z* 342.2198 (calcd for $C_{22}H_{30}O_3$, 342.2193).

5-Methyl-2-[(2′*E*,7′*Z*)-3′,7′,11′-trimethyl-2′,7′-dodecadien-9′-onyl]benzo-1,4-quinone (22): bright yellow oil; λ_{max} (MeOH) nm (log ϵ) 250 (3.45); IR ν_{max} 1682, 1657, 1615, 1445, 1384, 1238, 1134, 909 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.90 (6H, d, J = 7 Hz, H_3-12′, H_3-13′), 1.56 (2H, m, H_2-5′), 1.62 (3H, s, H_3-15′), 1.85 (3H, d, J = 1 Hz, H_3-14′), 2.01 (3H, d, J = 2 Hz, H_3-7), 2.08 (2H, t, J = 7 Hz, H_2-4′), 2.11 (1H, m, J = 7 Hz, H-11′), 2.25 (2H, d, J = 7 Hz, H_2-10′), 2.51 (2H, t, J = 8 Hz, H_2-6′), 3.10 (2H, d, J = 7 Hz, H_2-1′), 5.15 (1H, t, J = 7 Hz, H-2′), 6.02 (1H, s, H-8′), 6.49 (1H, d, J = 2 Hz, H-3), 6.57 (1H, d, J = 2 Hz, H-6) ppm; ^{13}C NMR data, see Table 2; EIMS *m/z* (rel int) 342 (12), 205 (18), 187 (13), 176 (16), 175 (100), 149 (20), 109 (11), 95 (13), 81 (10); HRFABMS *m/z* 343.2273 (calcd for $C_{22}H_{31}O_3$, 343.2273).

5-Methyl-2-[(2′*E*,6′*E*)-3′,7′,11′-trimethyl-2′,6′-dodecadien-9′-onyl]-1,4-dihydroxybenzene (23): orange oil; λ_{max} (MeOH) nm (log ϵ) 295 (3.27); IR ν_{max} 3396, 1698, 1420, 1368, 1192, 1003, 872 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.89 (6H, d, J = 7 Hz, H_3-12′ H_3-13′), 1.63 (3H, s, H_3-14′), 1.69 (3H, s, H_3-15′), 2.11 (3H, m, H_2-4, H-11′), 2.16 (3H, s, H_3-7), 2.19 (2H, m, H_2-5′), 2.31 (2H, d, J = 6 Hz, H_2-10′), 3.03 (2H, s, H_2-8′), 3.26 (2H, d, J = 7 Hz, H_2-1′), 4.83 (1H, br s, O*H*-1), 5.19 (1H, t, J = 6 Hz, H-6′), 5.28 (1H, t, J = 7 Hz, H-2′), 5.72 (1H, br s, O*H*-4), 6.54 (1H, s, H-3), 6.56 (1H, s, H-6) ppm; ^{13}C NMR data, see Table 2; EIMS *m/z* (rel int) 344 (59), 244 (27), 215 (21), 189 (41), 177 (29), 176 (23), 175 (100), 161 (13), 137 (30); HRFABMS *m/z* 344.2351 (calcd for $C_{22}H_{32}O_3$, 344.2352).

5-Methyl-2-[(2′*E*,7′*Z*)-3′,7′,11′-trimethyl-2′,7′-dodecadien-9′-onyl]-1,4-dihydroxybenzene (24): orange oil; λ_{max} (MeOH) nm (log ϵ) 294 (3.19), 232 (4.31); IR ν_{max} 3369, 1667, 1603, 1420, 1380, 1192, 873 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.92 (6H, d, J = 7 Hz, H_3-12′, H_3-13′); 1.61 (2H, m, H_2-5′); 1.66 (3H, s, H_3-15′); 1.90 (3H, s, H_3-14′); 2.14 (1H, m, H-11′); 2.16 (2H, m, H_2-4′); 2.17 (3H, s, H_3-7); 2.29 (2H, d, J = 7 Hz, H_2-10′); 2.68 (2H, t, J = 8 Hz, H_2-6′); 3.27 (2H, d, J = 8 Hz, H_2-1′); 4.54 (1H, s, O*H*-4); 5.35 (1H, t, J = 7 Hz, H-2′); 6.08 (1H, s, H-8′); 6.55 (1H, s, H-6); 6.83 (1H, s, H-3); 6.96 (1H, s, O*H*-1) ppm; EIMS *m/z* (rel int) 344 (54), 205 (30), 189 (86), 177 (33), 176 (35), 175 (100), 137 (31), 133 (29), 91 (27), 81 (38); ^{13}C NMR data, see Table 2; HRFABMS *m/z* 344.2351 (calcd for $C_{22}H_{32}O_3$, 344.2352).

1-Acetoxy-5-methyl-2-[(2′*E*,7′*Z*)- 3′,7′,11′-trimethyl-2′,7′-dodecadien-9′-onyl]-4-hydroxybenzene (25): yellow oil; λ_{max} (MeOH) nm (log ϵ) 281 (3.52), 242 (4.11); IR ν_{max} 3369, 1760, 1667, 1607, 1445, 1368, 1214, 1180, 1010, 915 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.92 (6H, d, J = 7 Hz, H_3-12′, H_3-13′), 1.61 (3H, s, H_3-15′), 1.63 (2H, m, H_2-5′), 1.64 (1H, m, H-11′), 1.92 (3H, s, H_3-14′), 2.17 (2H, m, H_2-4′), 2.19 (3H, s, H_3-7), 2.27 (3H, s, Ac-Me), 2.30 (2H, d, J = 7 Hz, H_2-10′), 2.72 (2H, br t, J = 9 Hz, H_2-6′), 3.13 (2H, d, J = 8 Hz, H_2-1′), 5.32 (1H, t, J = 7 Hz, H-2′), 6.10 (1H, s, H-8′), 6.73 (1H, s, H-6), 7.01 (1H, s, H-3), 8.13 (1H, s, O*H*-4) ppm; ^{13}C NMR data, see Table 2; EIMS *m/z* (rel int) 386 (18), 344 (30), 227 (100), 189 (51), 175 (44), 149 (45), 111 (44), 95 (65), 81 (56), 67 (43); HRFABMS *m/z* 386.24567 (calcd for $C_{24}H_{34}O_4$, 386.24571).

Chromenol (26): orange oil; λ_{max} (MeOH) nm (log ϵ) 331 (3.11), 267 (3.68), 222 (4.43); IR ν_{max} 3404, 1702, 1459, 1368, 1178, 1005, 919, 873 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.88 (6H, d, J = 7 Hz, H_3-12′, H_3-13′), 1.34 (3H, s, H_3-15′), 1.57 (3H, s, H_3-14′), 1.68 (2H, m, H_2-4′), 2.10 (2H, m, H_2-5′), 2.17 (3H, s, H_3-7), 2.26 (2H,d, J = 7 Hz, H_2-10′), 2.98 (2H, s, H_2-8′), 4.41 (1H, br s, O*H*-4), 5.23 (1H, t, J = 7 Hz, H-6′), 5.51 (1H, d, J = 10 Hz, H-2′), 6.24 (1H, d, J = 10 Hz, H-1′), 6.41 (1H, s, H-3), 6.54 (1H, s, H-6) ppm; ^{13}C NMR data, see Table 2; EIMS *m/z* (rel int) 342 (12), 325 (12), 300 (16), 299 (48), 283 (18), 281 (34), 227 (25), 225 (25), 211 (25), 209 (27); HRFABMS *m/z* 342.2194 (calcd for $C_{22}H_{30}O_3$, 342.2195).

5-Methyl-2-[(2′*E*,6′*E*)-9′-hydroxy-3′,7′,11′-trimethyl-2′,6′-dodecadienyl]-1,4-dihydroxybenzene (27): orange oil; λ_{max} (MeOH) nm (log ϵ) 294 (3.24); IR ν_{max} 3369, 1421, 1381, 1191, 872 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.93 (6H, d, J = 7 Hz, H_3-12′, H_3-13′), 1.22 (1H, m, H-10′), 1.25 (1H, s, O*H*-9′), 1.49 (1H, m, H-10′), 1.64 (3H, s, H_3-14′), 1.67 (3H, s, H_3-15′), 1.82 (1H, sept, J = 7 Hz, H-11′), 1.96 (1H, dd, J = 3, 10 Hz, H-8′), 2.16 (3H, s, H_3-7), 2.18 (3H, m, H_2-4, H-5′), 2.21 (1H, dm, J = 16 Hz, H-8′), 2.29 (1H, pent, J = 8 Hz, H-5′), 3.26 (2H, d, J = 7 Hz, H_2-1′), 3.76 (1H, br m, H-9′), 4.86 (1H, br s, O*H*-1), 5.24 (1H, m, H-6′), 5.27 (1H, m, H-2′), 5.29 (1H, br s, O*H*-4), 6.45 (1H, s, H-3), 6.57 (1H, s, H-6) ppm; ^{13}C NMR data, see Table 2; EIMS *m/z* (rel int) 344 (10), 326 (13), 278 (8), 241 (8), 213 (9), 189 (8), 176 (16), 175 (100), 105 (10), 91 (11); HRFABMS *m/z* 346.2508 (calcd for $C_{22}H_{34}O_3$, 346.2508).

Hydrogenation of Cubebenone (8). Hydrogenation of **8** (9.0 mg) over a Pd−C catalyst (20 mg) in ethanol gave **11** as a yellow oil; $[\alpha]^{20}_D$ +65° (*c* 0.35, $CHCl_3$); IR ν_{max} 1719, 1456, 1370, 1256, 1023, 870 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.49 (1H, qd, J = 2, 12 Hz, H-2), 0.82 (1H, qd, J = 2, 13 Hz, H-3), 0.87 (3H, d, J = 6 Hz, H_3-14), 0.92 (3H, d, J = 7 Hz, H_3-13), 0.97 (3H, d, J = 7 Hz, H_3-12), 1.04 (1H, m, H-4), 1.07 (1H, t, J = 4 Hz, H-10), 1.12 (3H, d, J = 7 Hz, H_3-15), 1.43 (1H, dd J = 2, 13 Hz, H-3), 1.61 (1H, m, J = 7 Hz, H-11), 1.67 (1H, t, J = 4 Hz, H-5), 1.68 (1H, d, J = 12 Hz, H-2), 1.73 (1H, q, J = 9 Hz, H-7), 2.13 (1H, q, J = 9 Hz, H-7), 2.43 (1H, m, H-6) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.2 (q, C-15), 19.9 (q, C-12/13), 19.9 (q, C-14), 20.2 (q, C-13/12), 25.3 (d, C-1), 26.7 (t, C-3), 27.1 (d, C-10), 29.6 (d, C-6), 30.6 (t, C-2), 33.6 (d, C-11), 37.6 (d, C-5), 40.9 (t, C-7), 44.7 (d, C-4), 45.0 (s, C-9), 215.9 (s, C-8) ppm; EIMS *m/z* (rel int) 220 (39), 177 (34), 149 (59), 135 (44), 109 (30), 97 (41), 57 (52), 55 (69), 43 (94), 41 (100); HREIMS *m/z* 220.1818 (calcd for $C_{15}H_{24}O$, 220.1826).

Attempted Thermal Degradation of Cubebenone (8). Compound **8** (2.5 mg) was sealed in a 1 mL glass ampule and

heated in a hot block (6 h, 135 °C). After 2 h, GC analysis of **8** showed that no **9** was present. The temperature was raised to 150 °C, and two further GC injections of the sample were made after 4 and 6 h with similar negative results.

Extraction of *L. millecra* and Octocoral Specimens for GC and LC Analyses. Eight specimens of *L. millecra* and three pieces of different gorgonians (*Leptogorgia* sp., *L. palma*, and an *Acabaria* species) were extracted separately in acetone, and the resulting crude acetone extracts were partitioned between water and EtOAc (3 × 20 mL) and dried to afford between 2 and 30 mg of concentrated crude extract from each specimen.

GC Analyses of Pure Compounds, Octocoral, and Nudibranch Extracts. Solutions (0.1 mg mL^{-1}) of each of the pure compounds **1** (t_R 20.17 min), **2** (25.25 min), **8** (21.50 min), **9** (24.92 min), and **26** (41.11 min), and a mixture of all five compounds, were analyzed by GC to provide standards for the analysis of crude octocoral and nudibranch extracts. EtOAc solutions (0.5 mg mL^{-1}) of 18 organic octocoral extracts provided by the NCI and extracts of the individual nudibranchs (× 8) and octocoral pieces collected (× 3) were filtered and qualitatively analyzed by GC for the presence of the five standard compounds. Millecrone A (**1**) was evident in NCI crude extracts OCDN 6174 and OCDN 6176. Each of the eight *L. millecra* specimens collected individually and the octocoral and *Leptogorgia palma* contained millecrone B (**2**) and cubebenone (**8**).

Acknowledgment. We would like to thank Pat and Lori Colin and the CRRF team and Anton Cloete and Shirley Kuiters of the UPE Research Diving Unit for assistance with the collection and photography of *L. millecra* and the octocorals of Algoa Bay. The assistance of Dr. Dave Newman and Dr. Gordon Cragg of the NCI in readily providing Algoa Bay octocoral extracts and identifications is also greatly appreciated. The financial support for this research from the National Research Foundation and Rhodes University (including the Rhodes University Scholarship provided for K.L.M.) is gratefully acknowledged.

References and Notes

(1) Gosliner, T. In *Nudibranchs of Southern Africa*; Behrens, D. W., Hashagen K., Eds.; Sea Challengers: Monterey, CA, 1987; pp 109−112.
(2) Pika, J.; Faulkner, D. J. *Tetrahedron* **1994**, *50*, 3065−3070.
(3) McPhail, K.; Davies-Coleman, M. T. *Tetrahedron* **1997**, *53*, 4655−4660. McPhail, K. L.; Davies-Coleman, M. T.; Coetzee, P. S. *J. Nat. Prod.* **1998**, *61*, 961−964. McPhail, K. L.; Davies-Coleman, M. T.; Copley, R. C. B.; Eggleston, D. S. *J. Nat. Prod.* **1999**, *62*, 1618−1623.
(4) Bowden, B. F.; Braekman, J. C.; Coll, J. C.; Mitchell, S. J. *Aust. J. Chem.* **1980**, *33*, 927−932.
(5) Hikino, H.; Agatsuma, K.; Takemoto, T. *Tetrahedron Lett.* **1968**, *8*, 931−933.
(6) Izac, R. R.; Bandurraga, M. M.; Wasylyk, J. M.; Dunn, F. W.; Fenical, W. *Tetrahedron* **1982**, *38*, 301−304.
(7) Chan, W. R.; Tinto, W. F.; Moore, R. *Tetrahedron* **1990**, *46*, 1499−1502.
(8) Cimino, G.; De Rosa, S.; De Stefano, S.; Sodano, G. *J. Nat. Prod.* **1984**, *47*, 877−878.
(9) Coll, J. C.; Mitchell, S. J.; Stokie, G. J. *Tetrahedron Lett.* **1977**, *18*, 1539−1542. Bowden, B. F.; Coll, J. C.; de Silva, E. D.; de Costa, M. S. L.; Djura, P. J.; Mahendran, M.; Tapiolas, D. M. *Aust. J. Chem.* **1983**, *36*, 371−376. Park, S. K.; Scheuer, P. J. *J. Korean Chem. Soc.* **1994**, *38*, 749−752.
(10) Bowden, B. F.; Coll, J. C.; Tapiolas, D. M. *Aust. J. Chem.* **1983**, *36*, 211−214.
(11) Henderson, G. G.; Robertson, J. M. *J. Chem. Soc.* **1926**, 2811−2816. Vonasek, F.; Herout, V.; Sorm, F. *Collect. Czech. Chem. Commun.* **1960**, *25*, 919−926. Tanaka, A.; Tanaka, R.; Uda, H.; Yoshikoshi, A. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1721−1727.
(12) Suzuki, M.; Kowata, N.; Kurosawa, E. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 2366−2368.
(13) Weinheimer, A. J.; Schmitz, F. J.; Ciereszko, L. S. *Trans. Marine Technol. Soc. Symp.* **1967**, 135−140.
(14) Kashman, Y. *Tetrahedron* **1979**, *35*, 263−266.
(15) Nagashima, F.; Momasaki, S.; Watanabe, Y.; Takaoka, S.; Huneck, S.; Asakawa, Y. *Phytochemistry* **1996**, *42*, 1361−1366. Warmers, U.; Konig, W. A. *Phytochemistry* **1999**, *52*, 99−104.
(16) Tanaka, J.; Nobutani, K.; Adachi, K. *Nippon Kagaku Kaishi* **1988**, *7*, 1065−1073 (CA 111: 115608g).
(17) Bowden, B. F.; Coll, J. C.; Engelhardt, L. M.; Tapiolas, D. M.; White, A. H. *Aust. J. Chem.* **1986**, *39*, 103−121.
(18) Hooper, G. J.; Davies-Coleman, M. T. *Tetrahedron Lett.* **1995**, *36*, 3265−3268.
(19) Fusetani, N.; Yasukawa, K.; Matsunaga, S.; Hashimoto, K. *Tetrahedron Lett.* **1985**, *26*, 6449−6452.
(20) Bowden, B. F.; Coll, J. C. *Aust. J. Chem.* **1981**, *34*, 2677−2681.
(21) Dorman, D. E.; Jautelat, M.; Roberts, J. D. *J. Org. Chem.* **1971**, *36*, 2757−2766.
(22) Baek, S.-H.; Perry, N. B.; Weavers, R. T.; Tangney, R. S. *J. Nat. Prod.* **1998**, *61*, 126−129.
(23) Koren-Goldschlager, G.; Klein, P.; Rudi, A.; Benayahu, Y.; Schleyer, M.; Kashman, Y. *J. Nat. Prod.* **1996**, *59*, 262−266.
(24) Kobayashi, M. *J. Chem. Res. Synop.* **1994**, 494−495.
(25) Shin, J.; Seo, Y.; Cho, K. W.; Moon, S.; Cho, Y. J. *J. Org. Chem.* **1999**, *64*, 1853−1858.
(26) Gerwick, W. H.; Fenical, W. *J. Org. Chem.* **1981**, *46*, 22−27.
(27) Faulkner, D. J. *Nat. Prod. Rep.* **2001**, *18*, 1−49, and previous reviews in this series.

NP010085X