

Antioxidative Meroterpenoids from the Brown Alga *Cystoseira crinita*

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Six new tetraprenyltoluquinol derivatives (**1–6**), two new triprenyltoluquinol derivatives (**7** and **8**), and two new tetraprenyltoluquinone derivatives (**9** and **10**) were isolated from the brown alga *Cystoseira crinita* Duby together with four known tetraprenyltoluquinol derivatives (**11–14**). All structures were elucidated by employing spectroscopic techniques (NMR, MS, UV, and IR). Each compound was evaluated for its antioxidative properties in the TBARS and DPPH assay, and compounds **1**, **2**, **6**, and **10–14** were additionally assessed in the TEAC and PCL assay. Hydroquinones were found to have powerful antioxidant activity.

Reactions of free radicals such as superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($\cdot OH$), peroxy radical (ROO^{\cdot}), nitric oxide (NO^{\cdot}), and other reactive oxygen and nitrogen species are associated with diseases such as atherosclerosis, dementia, and cancer.^{1,2} Lipids, proteins, and DNA are the targets of such species and undergo oxidative reactions leading to their degradation. An antioxidant is “any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate”.³

Phenols are particularly effective antioxidants for polyunsaturated fatty acids because they can easily transfer a hydrogen atom to lipid peroxy radicals. The aryloxy radical formed in this reaction is usually too “sluggish” to act as a chain carrier, so that the chain reaction of lipid peroxidation is interrupted.

To date there are only a few investigations using different methods to determine antioxidant properties of naturally occurring antioxidants. These studies mostly assess hydrophilic antioxidants, while investigations on lipophilic antioxidants are scarce.^{4–8} From the numerous assays that exist to detect antioxidative activity in vitro the Thiobarbituric Acid Reactive Substances (TBARS) method was chosen in order to detect antioxidants that can prevent linolenic acid methyl ester from being oxidized. Reaction with the α, α -diphenyl- β -picrylhydrazyl radical (DPPH) served as a method for the direct detection of radical-scavenging activity in organic solutions. Both systems were established as screening methods also suitable for bioassay-guided fractionation; the assays were performed in microtiter plates, and activities were assessed by measuring characteristic absorbances. Additionally, two further test systems, i.e., Trolox Equivalent Antioxidant Capacity (TEAC) and Photochemiluminescence (PCL) assay, were used to analyze some of the pure compounds for their antioxidant potential. TEAC and PCL assay also determine the radical-scavenging capacities of antioxidants and are applicable for lipophilic compounds.

Screening algal extracts (DCM and MeOH extracts) for their antioxidative activity revealed the DCM extract obtained from *Cystoseira crinita* Duby to have prominent activity in the TBARS and the DPPH assay. Brown algae

of the genus *Cystoseira* are known to produce tetraprenyltoluquinol derivatives as characteristic secondary metabolites.⁹ Considering that tocopherols, very important natural antioxidants, are tetraprenyltoluquinol derivatives, as well as the fact that some of these algal metabolites, previously tested as antioxidants, showed activity comparable to that of α -tocopherol,¹⁰ the extract was further investigated in order to identify the active components.

Results and Discussion

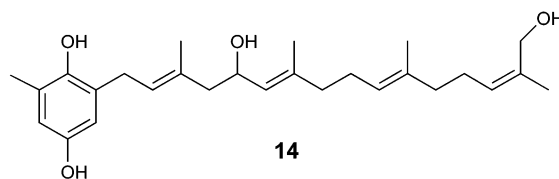
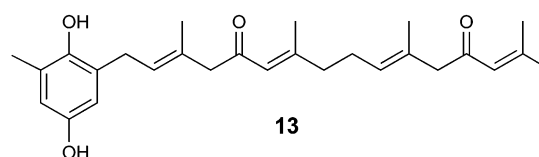
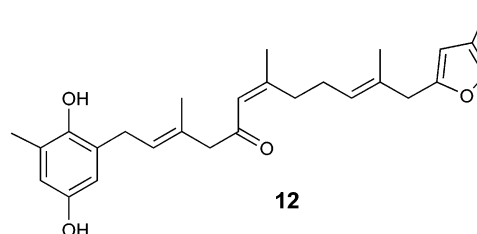
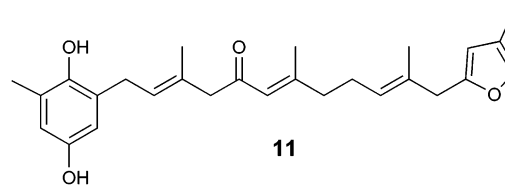
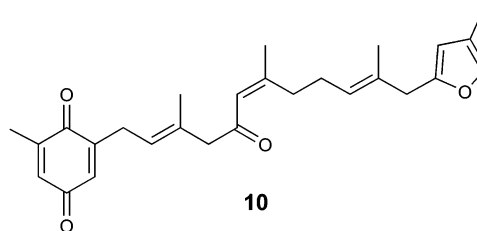
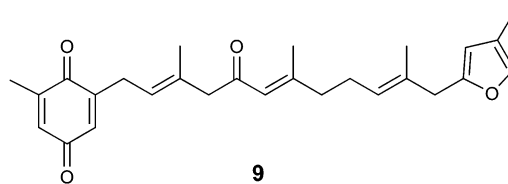
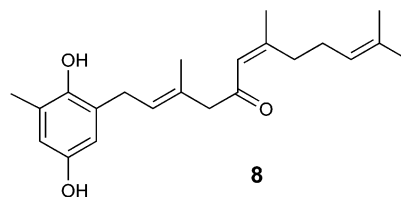
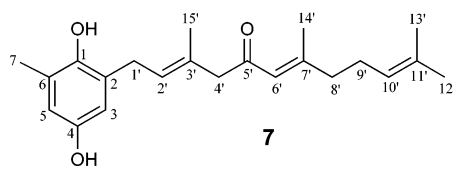
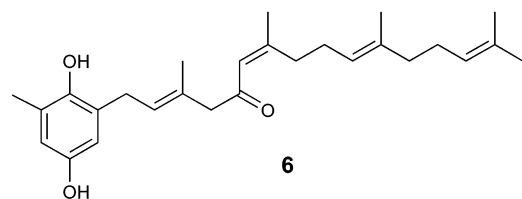
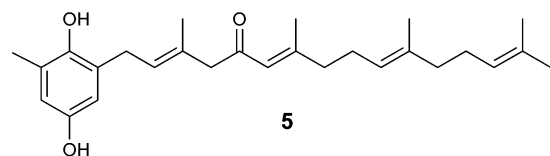
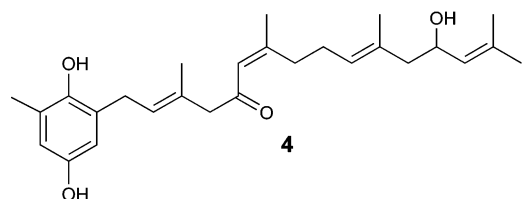
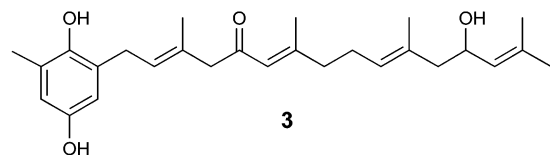
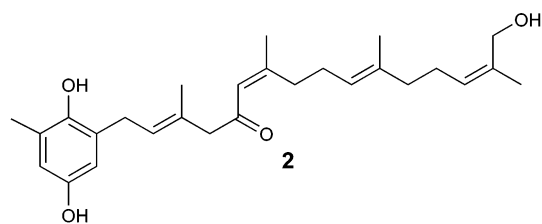
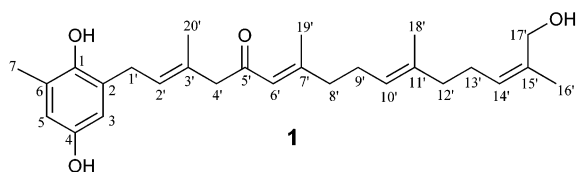
The current sample of *Cystoseira crinita* was collected from the south coast of Sardinia and stored in ethanol at $-4^\circ C$ until workup. After extraction with MeOH and DCM the extracts were evaluated for their biological activities. Simultaneous with these assays, investigation of the secondary metabolite chemistry of the algal sample was started. Chromatographic separation of the acetone-soluble part of the MeOH extract yielded six new tetraprenyltoluquinol derivatives (**1–6**), two new triprenyltoluquinol derivatives (**7** and **8**), two new tetraprenyltoluquinone derivatives (**9** and **10**), and the known tetraprenyltoluquinol derivatives 5-oxo-cystofuranoquinol (**11**), 5-oxo-isocystofuranoquinol (**12**), 2-[(2'*E*,6'*E*,10'*E*)-5',13'-dioxo-3',7',11',15'-tetramethylhexadeca-2',6',10',14'-tetraenyl]-6-methylhydroquinone (**13**), and 2-[(2'*E*,6'*E*,10'*E*,14'*Z*)-5'-hydroxy-15'-hydroxymethyl-3',7',11'-trimethylhexadeca-2',6',10',14'-tetraenyl]-6-methylhydroquinone (**14**), previously isolated from *Cystoseira spinosa* var. *squarrosa*.¹¹

Compound **1** has the molecular formula $C_{27}H_{38}O_4$ (HR-EIMS), indicating it to have 9 degrees of unsaturation. Its UV spectrum was consistent with the presence of a hydroquinol moiety [λ_{max} 236 nm ($\epsilon = 12\,100$), 290 nm ($\epsilon = 3400$)]. The ^{13}C NMR data of **1** contained a total of 27 resonances for five methyl, seven methylene, and six methine groups, and nine quaternary carbons, including a carbonyl group (δ 199.0) and as seen in the DEPT spectrum of **1** a hydroxymethylene group (δ 61.0). The 1H NMR spectrum (Table 1) measured in acetone- d_6 showed signals for two aromatic protons (δ 6.45, 2H) as well as a benzylic methylene group (δ 3.32, d, $J = 7.3$ Hz, H-1') that coupled with the olefinic proton H-2' (δ 5.44, brt, $J = 7.3$ Hz). In addition, two protons of aromatic OH groups (δ 6.56, 1-OH and δ 7.54, 4-OH) were observed as sharp singlets. A 1H - ^{13}C 2D NMR shift correlated measurement (HMBC) of **1** showed long-range couplings between 1-OH and C-1, C-2, and C-6 and between 4-OH and C-4, C-3, and C-5. These observa-

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tions together with a long-range HMBC coupling of H₃-7 to C-1, C-2, C-5, and C-6 clarified the structure of the aromatic part of **1** as 6-methylhydroquinone. HMBC correlations between H₂-1' and C-2, C-2', C-1, C-3, and C-3', and between H₃-20' and C-2', C-3', and C-4', were consistent with an isoprene unit being attached to the aromatic moiety. The deshielded nature of the signals associated with H₂-4', H₃-19', and H-6' in the ¹H NMR spectrum (Table 1) revealed the proximity of these atoms to a conjugated carbonyl function (C-5', δ 199.0), and heteronuclear long-range couplings between H-6' and C-5', C-8', and C-19' allowed the already developed partial structure of **1** to be extended to include a second and carbonyl-containing isoprene residue. The chemical shifts and multiplicities of the as yet unassigned C atoms showed that the remaining two degrees of unsaturation, indicated by the molecular formula of **1**, were present as two C=C double bonds in an acyclic isoprenoid side chain. From the ¹H NMR and ¹³C NMR spectrum of **1** it was evident that one of the anticipated five methyl groups present in an acyclic tetraprenyl chain was present as a hydroxymethylene group (H₂, δ 4.05, d, *J* = 5.5 Hz; OH, δ 3.58, t, *J* = 5.5 Hz; C-17', δ 61.0). Long-range ¹H-¹³C correlations between the resonance for H₂-17' and that for C-16' showed the hy-

droxymethylene to be part of the terminal isoprenoid unit. The remaining isoprenoid unit was assigned from the heteronuclear long-range couplings seen between H₂-12' and C-13', C-14', C-10', and C-11', thus establishing the planar structure of **1**. The geometry of the four double bonds was assigned on the basis of the ¹³C NMR chemical shifts for CH₃-20', CH₃-19', CH₃-18', and CH₃-16'. Thus,

Table 1. ¹H NMR Spectral Data for Compounds **1–8** (δ ppm, mult., *J* in Hz)^a

carbon	1 ^b	2 ^b	3 ^b	4 ^b	5 ^b	6 ^b	7 ^c	8 ^c
3	6.45 (1H, m)	6.45 (1H, m)	6.46 (1H, d, 2.8)	6.45 (1H, m)	6.45 (1H, m)	6.45 (1H, m)	6.46 (1H, d, 3.0)	6.45 (1H, m)
5	6.45 (1H, m)	6.45 (1H, m)	6.44 (1H, d, 2.8)	6.45 (1H, m)	6.45 (1H, m)	6.45 (1H, m)	6.44 (1H, d, 3.0)	6.45 (1H, m)
7	2.16 (3H, m)	2.16 (3H, m)	2.15 (3H, s)	2.15 (3H, m)	2.15 (3H, m)	2.15 (3H, m)	2.15 (3H, s)	2.16 (3H, s)
1'	3.32 (2H, d, 7.3)	3.32 (2H, d, 7.3)	3.32 (2H, d, 7.3)	3.33 (2H, d, 7.3)	3.32 (2H, d, 7.3)	3.32 (2H, d, 7.3)	3.33 (2H, d, 7.3)	3.33 (2H, d, 7.3)
2'	5.44 (1H, brt, 7.3)	5.44 (1H, brt, 7.3)	5.43 (1H, brt, 7.3)	5.43 (1H, brt, 7.3)	5.43 (1H, brt, 7.3)	5.44 (1H, brt, 7.3)	5.44 (1H, brt, 7.3)	5.44 (1H, brt, 7.3)
4'	3.10 (2H, s)	3.10 (2H, s)	3.10 (2H, s)	3.08 (2H, s)	3.10 (2H, s)	3.08 (2H, s)	3.09 (2H, s)	3.08 (2H, s)
6'	6.19 (1H, s)	6.19 (1H, s)	6.19 (1H, s)	6.19 (1H, s)	6.19 (1H, s)	6.19 (1H, s)	6.19 (1H, s)	6.19 (1H, s)
8'	2.16 (2H, m)	2.58 (2H, t, 7.8)	2.15 (2H, m)	2.58 (2H, t, 7.5)	2.15 (2H, m)	2.58 (2H, t, 7.7)	2.14 (2H, m)	2.56 (2H, t, 7.9)
9'	2.16 (2H, m)	2.16 (2H, m)	2.16 (2H, m)	2.16 (2H, m)	2.15 (2H, m)	2.15 (2H, m)	2.15 (2H, m)	2.10 (2H, m)
10'	5.11 (1H, brt, 4.8)	5.17 (1H, brt, 6.2)	5.14 (1H, m)	5.18 (1H, brt, 7.0)	5.11 (1H, m)	5.16 (1H, brt, 7.3)	5.09 (1H, t, 6.7)	5.13 (1H, t, 7.3)
12'	1.97 (2H, m)	1.97 (2H, m)	2.16 (1H, m) 2.05 (1H, m)	2.15 (1H, m) 2.06 (1H, m)	1.97 (2H, m)	1.97 (2H, m)	1.65 (3H, s)	1.64 (3H, s)
13'	2.16 (2H, m)	2.16 (2H, m)	4.40 (1H, m)	4.41 (1H, m)	2.06 (2H, m)	2.06 (2H, m)	1.58 (3H, s)	1.60 (3H, s)
14'	5.18 (1H, brt, 7.0)	5.18 (1H, brt, 7.0)	5.11 (1H, m)	5.11 (1H, d, 8.0)	5.08 (1H, m)	5.09 (1H, brt, 7.0)	2.09 (3H, s)	1.86 (3H, s)
15'							1.70 (3H, s)	1.69 (3H, s)
16'	1.72 (3H, s)	1.73 (3H, s)	1.65 (3H, s)	1.65 (3H, s)	1.64 (3H, s)	1.64 (3H, s)		
17'	4.05 (2H, d, 5.5)	4.05 (2H, d, 5.5)	1.61 (3H, s)	1.62 (3H, s)	1.57 (3H, s)	1.58 (3H, s)		
18'	1.59 (3H, s)	1.61 (3H, s)	1.62 (3H, s)	1.65 (3H, s)	1.59 (3H, s)	1.61 (3H, s)		
19'	2.09 (3H, s)	1.88 (3H, s)	2.09 (3H, s)	1.89 (3H, s)	2.09 (3H, s)	1.89 (3H, s)		
20'	1.69 (3H, s)	1.70 (3H, s)	1.69 (3H, s)	1.69 (3H, s)	1.69 (3H, s)	1.69 (3H, s)		
1-OH	6.56 (1H, s)	6.56 (1H, s)	6.56 (1H, s)	6.56 (1H, s)	6.56 (1H, s)	6.57 (1H, s)	6.48 (1H, s)	6.52 (1H, s)
4-OH	7.54 (1H, s)	7.54 (1H, s)	7.54 (1H, s)	7.54 (1H, s)	7.55 (1H, s)	7.55 (1H, s)	7.47 (1H, s)	7.48 (1H, s)
13'-OH			3.26 (1H, d, 4.0)	3.21 (1H, d, 4.0)				
17'-OH	3.58 (1H, t, 5.5)	3.58 (1H, t, 5.5)						

^a Assignments are based on 1D and 2D NMR measurements (HMBC, HMQC, COSY). ^b Acetone-*d*₆, 300 MHz. ^c Acetone-*d*₆, 500 MHz.

the ¹³C NMR resonances of CH₃-20', CH₃-19', and CH₃-18' (δ 16.6, 19.2, and 16.1, respectively) showed Δ^{2'}, Δ^{6'}, and Δ^{10'} to have the *E* geometry, whereas that of CH₃-16' (δ 21.5) indicated Δ^{14'} to have the *Z* geometry.

The second isolated compound, **2**, was an optically inactive oil and isomeric with compound **1**. Its UV and IR data [λ_{max} 236 nm (ε = 13 000), 285 nm (ε = 4200); IR (film) ν_{max} 3330, 2919, 1674, 1607, 1470 cm⁻¹] resembled those of **1**, with the mass spectral fragmentation pattern of the two compounds being almost identical. The ¹H NMR spectrum of **2** (Table 1), when compared with that of **1**, displayed differences that were attributable to the partial structure influenced by the geometry of Δ^{6'}. Thus the chemical shift of H₃-19' shifted from δ 2.09 in **1** to δ 1.88 in **2**, and the H₂-8' methylene resonance shifted downfield from δ 2.16 in **1** to δ 2.58 in **2**, consistent with a change in the geometry of the Δ^{6'} double bond from *E* in **1** to *Z* in **2**. The same observations were made for the known compounds **11** and **12** (see Table 2) and also in the literature.¹¹ Analogously, in the ¹³C NMR spectrum of **2** (Table 3) the resonances associated with C-8' and C-19' were the only ones whose chemical shifts differed significantly from those of the corresponding atoms in **1**. The ¹³C NMR chemical shift of C-19' (δ 25.6) is consistent with the *Z* geometry of Δ^{6'}.

Compound **3** was obtained as an optically active oil, [α]_D²⁸ +4.6°. Its EIMS spectrum contained a weak signal at *m/z* 426 corresponding to the molecular ion. HREIMS of *m/z* 408 [M - H₂O]⁺ led to C₂₇H₃₈O₄ as the molecular formula of **3**. The intense fragment ion in the MS at *m/z* 175 indicated the presence of a partial structure consisting of a toluquinol moiety, having an attached isoprene unit.¹¹ Chemical shifts in ¹H and ¹³C NMR spectra (Tables 1 and

Table 2. ¹H NMR Spectral Data for Compounds **9–11** (δ ppm, mult., *J* in Hz)

carbon	9 ^a	10 ^a	11 ^b
3	6.52 (1H, m)	6.51 (1H, m)	6.45 (1H, m)
5	6.59 (1H, m)	6.59 (1H, m)	6.45 (1H, m)
7	2.01 (3H, s)	2.01 (3H, s)	2.16 (3H, s)
1'	3.18 (2H, d, 7.3)	3.17 (2H, d, 7.3)	3.33 (2H, d, 7.3)
2'	5.36 (1H, brt, 7.3)	5.37 (1H, brt, 7.3)	5.44 (1H, brt, 7.3)
4'	3.15 (2H, s)	3.14 (2H, s)	3.10 (2H, s)
5'			
6'	6.19 (1H, s)	6.20 (1H, s)	6.19 (1H, s)
8'	2.16 (2H, m)	2.60 (2H, t, 7.8)	2.16 (2H, m)
9'	2.21 (2H, m)	2.17 (2H, m)	2.16 (2H, m)
10'	5.22 (1H, brt, 6.6)	5.26 (1H, brt, 7.0)	5.21 (1H, brt, 7.3)
12'	3.21 (2H, s)	3.20 (2H, s)	3.20 (2H, s)
13'			
14'	5.91 (1H, s)	5.92 (1H, s)	5.90 (1H, s)
16'	7.13 (1H, s)	7.13 (1H, s)	7.13 (1H, s)
17'	1.93 (3H, s)	1.94 (3H, s)	1.94 (3H, s)
18'	1.58 (3H, s)	1.60 (3H, s)	1.57 (3H, s)
19'	2.10 (3H, s)	1.89 (3H, s)	2.09 (3H, s)
20'	1.65 (3H, s)	1.64 (3H, s)	1.69 (3H, s)
1-OH			6.57 (1H, s)
4-OH			7.55 (1H, s)

^a Acetone-*d*₆, 500 MHz. ^b Acetone-*d*₆, 300 MHz.

3) for this part of the molecule and for a second isoprene unit (C-5' to C-8') were almost identical to those found for compound **1**. The remaining signals in the ¹H and ¹³C NMR spectra of **3** (Tables 1 and 3) showed the molecule to contain two further isoprene units, one of which was hydroxylated (C-13' δ 67.3, H-13' δ 4.40 (m), 13'-OH δ 3.26 (d), *J* = 4 Hz). The ¹H-¹H COSY spectrum of **3** evidenced the hydroxymethine proton to be coupled with the 13'-OH

Table 3. ^{13}C NMR Spectral Data for Compounds **1–8** (δ ppm)^a

carbon	1 ^b	2 ^b	3 ^{b,d}	4 ^b	5 ^b	6 ^b	7 ^{c,d}	8 ^{c,d}
1	146.4 (s) ^e	146.4 (s)	146.4 (s)	146.4 (s)	146.4 (s)	146.4 (s)	146.4 (s)	146.3 (s)
2	129.5 (s)	129.5 (s)	129.5 (s)	129.6 (s)	129.5 (s)	129.5 (s)	129.5 (s)	129.2 (s)
3	114.4 (d)	114.5 (d)	114.5 (d)	114.5 (d)	114.5 (d)	114.5 (d)	114.5 (d)	114.3 (d)
4	151.3 (s)	151.3 (s)	151.3 (s)	151.3 (s)	151.3 (s)	151.3 (s)	151.4 (s)	151.2 (s)
5	115.7 (d)	115.7 (d)	115.7 (d)	115.7 (d)	115.7 (d)	115.7 (d)	115.7 (d)	115.4 (d)
6	126.3 (s)	126.4 (s)	not observed	126.3 (s)	126.3 (s)	126.3 (s)	126.4 (s)	126.3 (s)
7	16.8 (q)	16.8 (q)	16.8 (q)	16.8 (q)	16.8 (q)	16.8 (q)	16.8 (q)	16.6 (q)
1'	29.6 (t)	29.5 (t)	29.6 (t)	29.6 (t)	29.6 (t)	29.6 (t)	29.5 (t)	29.4 (t)
2'	128.1 (d)	128.1 (d)	128.2 (d)	128.2 (d)	128.1 (d)	128.2 (d)	128.1 (d)	128.0 (d)
3'	131.4 (s)	131.4 (s)	131.4 (s)	131.4 (s)	131.4 (s)	131.4 (s)	131.4 (s)	131.2 (s)
4'	55.8 (t)	55.8 (t)	55.8 (t)	55.8 (t)	55.8 (t)	55.8 (t)	55.8 (t)	55.6 (t)
5'	199.0 (s)	198.7 (s)	199.0 (s)	198.7 (s)	199.0 (s)	198.6 (s)	199.0 (s)	198.5 (s)
6'	123.4 (d)	123.9 (d)	123.4 (d)	124.0 (d)	123.4 (d)	123.9 (d)	123.4 (d)	123.8 (d)
7'	158.3 (s)	159.3 (s)	158.3 (s)	159.2 (s)	158.3 (s)	159.2 (s)	158.3 (s)	158.9 (s)
8'	41.5 (t)	34.2 (t)	41.5 (t)	34.1 (t)	41.5 (t)	34.2 (t)	41.6 (t)	34.2 (t)
9'	26.8 (t)*	27.3 (t)*	26.8 (t)	27.4 (t)	26.7 (t)	27.4 (t)	26.8 (t)	27.4 (t)
10'	124.2 (d)	124.8 (d)	126.8 (d)	127.2 (d)	124.1 (d)	124.6 (d)	124.2 (d)	124.7 (d)
11'	136.2 (s)	136.0 (s)	133.8 (s)	133.5 (s)	136.4 (s)	136.1 (s)	132.6 (s)	132.2 (s)
12'	40.6 (t)	40.7 (t)	49.1 (t)	49.2 (t)	40.4 (t)	40.4 (t)	25.8 (q)	25.5 (q)
13'	26.6 (t)*	26.8 (t)*	67.3 (d)	67.3 (d)	27.4 (t)	27.4 (t)	17.7 (q)	17.5 (q)
14'	126.8 (d)	126.9 (d)	130.2 (d)	130.3 (d)	125.0 (d)	125.1 (d)	19.1 (q)	25.3 (q)
15'	136.4 (s)	136.4 (s)	132.9 (s)	132.8 (s)	131.7 (s)	131.6 (s)	16.6 (q)	16.4 (q)
16'	21.5 (q)	21.5 (q)	25.8 (q)	25.8 (q)	25.8 (q)	25.8 (q)		
17'	61.0 (t)	61.1 (t)	18.2 (q)	18.2 (q)	17.7 (q)	17.7 (q)		
18'	16.1 (q)	16.0 (q)	16.6 (q)	16.6 (q)	16.1 (q)	16.0 (q)		
19'	19.2 (q)	25.6 (q)	19.2 (q)	25.5 (q)	19.2 (q)	25.5 (q)		
20'	16.6 (q)	16.6 (q)	16.7 (q)	16.6 (q)	16.6 (q)	16.5 (q)		

^a Assignments are based on 1D and 2D NMR measurements (HMBC, HMQC, COSY). Assignments marked with an asterisk (*) may be reversed. ^b Acetone-*d*₆, 75.5 MHz. ^c Acetone-*d*₆, 125 MHz. ^d Chemical shift values obtained from cross-peaks in HMBC and HMQC spectra. ^e Implied multiplicities determined by DEPT (C = s; CH = d; CH₂ = t; CH₃ = q).

proton, a methylene (H₂-12'), and an olefinic proton (H-14'). The latter proton was also coupled homoallylically to the protons of two methyl groups (H₃-16' and H₃-17'), indicating the hydroxyl group to be located at C-13' of the terminal isoprene unit. The unaffected chemical shift and coupling constant associated with the resonance of H₂-8' in compound **3** as compared to those values for the equivalent resonance of compound **1** also supported the position of the hydroxyl group.

HREIMS and ¹H and ¹³C NMR analysis of compound **4** showed it to have the molecular formula C₂₇H₃₈O₄. Comparison of its ¹H and ¹³C NMR data with those for **1–3** clearly showed it to be the Δ^6 *Z* isomer of **3**.

The molecular formula of **5** was deduced by accurate mass measurement to be C₂₇H₃₈O₃. Comparison of the ¹H and ¹³C NMR spectral data (Tables 1 and 3) of **5** with those of **1** and **3** made it clear that **5** was the 13'-dehydroxy derivative of **3**.

Compound **6**, molecular formula C₂₇H₃₈O₃ (HREIMS), was obtained as an optically inactive oil. Close comparison of its ¹H and ¹³C NMR data (see Tables 1 and 3) with those of **5** showed it to be the Δ^6 *Z* isomer of **5**.

Accurate mass measurement of compound **7** showed it to have the molecular formula C₂₂H₃₀O₃. Thus, compared to compound **5**, compound **7** has the equivalent of an isoprene unit (68 amu) less. Comparison of ¹H and ¹³C NMR data (see Tables 1 and 3) for **7** with those of **5** confirmed it to be a triprenyltoluquinol derivative with two unsubstituted isoprene units in the side chain instead of three such units as found in **5**. In all other respects the two molecules were identical, including the Δ^2 and Δ^6 carbon-carbon double bond geometries.

Compound **8** has the molecular formula C₂₂H₃₀O₃ (HREIMS). Comparison of the ¹H and ¹³C NMR data (see Tables 1 and 3) with those of **7** clearly showed it to be the Δ^6 *Z* isomer of **7**.

The molecular formula of compound **9** was established as C₂₇H₃₂O₄ by accurate mass measurement. Its IR spec-

trum showed the presence of a carbonyl functionality. The UV spectrum of **9** had a single maximum at 250 nm ($\epsilon = 25\,500$), consistent with an enone and a furan moiety, comparable to that of the known compound 5-oxo-cystofuranquinol (**11**), but no additional maxima for an aromatic hydroquinol. The ¹H NMR spectrum of **9** (see Table 2) was very similar to that of **11**, except that the signals for the OH protons were absent, and signals attributable to H-3 and H-5 were shifted downfield to δ 6.59 for H-5 and δ 6.52 for H-3 in **9** as compared to δ 6.45 (2H) in **11**. Additionally, the ¹H NMR resonance of H₂-1' shifted from δ 3.33 in **11** to δ 3.18 in **9**. HMBC correlations between the resonances for H₃-7 and those for C-5, C-6, and C-1 together with the characteristic ¹³C NMR chemical shift of C-1 (δ 188.1) evidenced the presence of a quinone moiety in **9** instead of the hydroquinone moiety found in **11**. The new compound **9** is best described as 5-oxo-cystofuranquinone.

Compound **10**, molecular formula C₂₇H₃₂O₄ (HREIMS), was obtained as an optically inactive oil. Comparison of its ¹H and ¹³C NMR data (see Tables 2 and 4) with those of **9** showed it to be the Δ^6 *Z* isomer of **9**, and thus to be best described as 5-oxo-isocystofuranquinone.

All of the compounds isolated in this study were tested for their antioxidative properties in the DPPH and TBARS assay. Results of these assays are given in Tables 5 and 6. In the DPPH test (Table 5) the hydroquinones showed a potent radical-scavenging effect comparable to that of α -tocopherol, that is, almost complete scavenging at a concentration of 230 μM (92.5–96.7% scavenging for compounds **1–8** and **11–14**, as compared to 95.2% scavenging for α -tocopherol) and still more than 10% scavenging at 23 μM . Differences in the values obtained in the DPPH assay for the individual compounds are probably due to small impurities of the samples, e.g., due to autoxidation and handling of small amounts rather than to really existing differences due to structural variations. The two quinones **9** and **10** showed activities significantly less than

Table 4. ^{13}C NMR Spectral Data for Compounds **9–11** (δ ppm)^a

carbon	9 ^{b,c}	10 ^{b,c}	11 ^d
1	188.1 (s) ^e	188.0 (s)	146.4 (s)
2	148.5 (s)	148.4 (s)	129.5 (s)
3	132.7 (d)	133.3 (d)	114.5 (d)
4	not observed	not observed	151.3 (s)
5	133.5 (d)	134.0 (d)	115.7 (d)
6	146.6 (s)	146.4 (s)	126.3 (s)
7	15.6 (q)	15.4 (q)	16.8 (q)
1'	28.0 (t)	28.0 (t)	29.6 (t)
2'	123.6 (d)	123.5 (d)	128.1 (d)
3'	134.8 (s)	134.7 (s)	131.4 (s)
4'	55.3 (t)	55.3 (t)	55.8 (t)
5'	198.3 (s)	197.8 (s)	199.0 (s)
6'	123.2 (d)	123.7 (d)	124.0 (d)
7'	158.1 (s)	159.1 (s)	158.1 (s)
8'	41.1 (t)	33.8 (t)	41.3 (t)
9'	26.7 (t)	27.2 (t)	26.7 (t)
10'	126.0 (d)	126.3 (d)	126.7 (d)
11'	133.4 (s)	132.9 (s)	133.4 (s)
12'	38.7 (t)	38.6 (t)	38.9 (t)
13'	154.7 (s)	154.7 (s)	155.0 (s)
14'	109.4 (d)	109.4 (d)	109.6 (d)
15'	121.1 (s)	120.9 (s)	121.2 (s)
16'	138.4 (d)	138.3 (d)	138.7 (d)
17'	9.6 (q)	9.6 (q)	9.8 (q)
18'	15.9 (q)	15.5 (q)	15.9 (q)
19'	19.0 (q)	25.3 (q)	19.2 (q)
20'	16.5 (q)	16.2 (q)	16.6 (q)

^a Assignments are based on 1D and 2D NMR measurements (HMBC, HMQC, COSY). ^b Acetone- d_6 , 125 MHz. ^c Chemical shift values obtained from cross-peaks in HMBC and HMQC spectra. ^e Implied multiplicities determined by DEPT (C = s; CH = d; CH₂ = t; CH₃ = q). ^d Acetone- d_6 , 75.5 MHz.

Table 5. DPPH^a Radical-Scavenging Activities of Compounds **1–14** Compared to BHT^b and α -Tocopherol

item tested	% scavenging ^c					
	6 ^d	12	23	58	115	230
1	8.0	12.3	25.1	64.8	96.1	96.4
2	9.1	15.2	31.2	80.1	96.0	96.7
3	4.6	7.4	18.1	49.2	93.2	96.1
4	5.8	9.7	17.1	55.2	95.5	95.5
5	6.0	8.6	24.4	67.4	95.0	94.4
6	6.9	11.8	25.5	67.2	95.4	95.4
7	2.6	3.7	11.2	29.8	59.1	94.1
8	2.6	5.9	17.7	50.2	92.1	92.5
9	1.0	2.2	2.9	8.1	16.0	29.0
10	1.1	1.8	5.5	12.2	22.8	38.6
11	8.0	12.5	24.9	65.4	95.2	95.4
12	5.9	11.0	22.1	56.7	95.7	95.8
13	7.6	14.3	29.1	80.8	95.7	95.5
14	7.4	16.2	30.7	79.2	95.6	95.7
BHT	1.2	2.9	5.6	11.8	22.0	35.6
α -tocopherol	8.2	16.3	33.9	88.1	94.9	95.2

^a DPPH = α,α -diphenyl- β -picrylhydrazyl. ^b BHT = butylated hydroxytoluene. ^c Scavenging % = $100 - (A \text{ sample}^e \times 100/A \text{ control}^e)$. ^d Concentrations in $\mu\text{mol/L}$. ^e Absorbance of sample and control measured at 517 nm.

α -tocopherol and the hydroquinols, but still comparable to that of butylated hydroxytoluene (BHT), i.e., 29.0% for **9** and 38.6% for **10** as compared to 35.6% scavenging observed for BHT at a concentration of 230 μM . At a concentration of 23 μM compound **9** showed 2.9% scavenging as compared to 5.5% for **10** and 5.6% for BHT.

The radical-scavenging activity of eight compounds (**1**, **2**, **6**, **10–14**) was further assessed using the TEAC and PCL assays (Table 6). These compounds showed activities between 13% (**11**) and 59% (**13**) that of α -tocopherol in the TEAC test and between 41% (**2**) and 112% (**10**) that of α -tocopherol in the PCL assay, indicating that they possessed potent radical-scavenging power. These results are

Table 6. Antioxidative Activity (TEAC^a and PCL^b Assay) of Compounds **1**, **2**, **6**, and **10–14** Compared to α -Tocopherol, Expressed as Trolox Equivalents in mmol/L^c

item tested	TEAC	PCL
1	0.09	0.59
2	0.09	0.51
6	0.14	1.35
10	0.30	1.41
11	0.08	1.06
12	0.28	0.79
13	0.37	1.39
14	0.09	0.72
α -tocopherol	0.63	1.26

^a TEAC = Trolox Equivalent Antioxidant Capacity. ^b PCL = photochemiluminescence. ^c Millimolar concentration of a Trolox solution having the antioxidant capacity equivalent to a 1.0 mM solution of the substance under investigation.

Table 7. Antioxidative Activities (TBARS Assay)^a of Compounds **1–14** Compared to BHT^b and α -Tocopherol

item tested	% inhibition ^c				
	8 ^d	16	33	82	164
1	10.6	14.8	32.1	67.2	73.7
2	12.0	18.3	31.5	69.8	73.4
3	7.8	11.7	17.6	55.4	68.9
4	4.9	10.4	19.2	60.4	70.3
5	13.3	17.0	24.3	61.7	70.8
6	10.7	12.1	28.6	66.8	71.8
7	9.2	19.8	22.2	43.1	66.8
8	13.2	19.9	28.8	52.9	66.5
9	7.6	14.2	20.2	31.4	43.3
10	11.1	16.1	24.4	38.7	54.4
11	15.1	25.8	41.2	69.3	74.9
12	16.4	25.2	38.9	66.6	74.6
13	13.6	20.3	39.9	70.1	72.2
14	9.0	22.3	35.3	67.5	71.1
BHT	23.1	35.4	49.1	63.3	69.3
α -tocopherol	13.1	32.1	67.6	71.2	72.7

^a TBARS = thiobarbituric acid method. ^b BHT = butylated hydroxytoluene. ^c % inhibition = $100 - (A \text{ sample}^e - A \text{ sample blank}) \times 100 / (A \text{ control} - A \text{ blank})$. ^d Concentrations in $\mu\text{mol/L}$. ^e A = absorbances are measured at 532 nm less the background at 600 nm.

consistent with those of the DPPH assay. Differences in the rank order of potency are dependent on the test system used. In the TEAC assay the rank order from the most active to the least active compound was **13** > **10** > **12** > **6** > **1** = **2** = **14** > **11**, while it was **10** \approx **13** > **6** > **11** > **12** > **14** > **1** > **2** in the PCL assay and **13** > **2** > **14** > **6** > **11** > **1** > **10** in the DPPH assay (based on a concentration of 58 $\mu\text{mol/L}$). Interestingly quinone **10** appears in the TEAC and in the PCL test among the compounds with the highest activities, in contrast to the results seen in the DPPH test. Similar observations concerning differences in test results when several assay systems have been used were made recently for four hydrophilic antioxidants, as well as for several beverages containing hydrophilic antioxidants.⁷

In the TBARS assay potent inhibition of peroxidation of linolenic acid methyl ester was observed for all hydroquinones, i.e., 66.5–74.9% inhibition for compounds **1–8** and **11–14** at a concentration of 164 μM (Table 7). These activities were comparable to both α -tocopherol (72.7%) and BHT (69.3%) at this concentration. A lower peroxidation-inhibiting activity for the compounds with a quinone moiety was obvious at high concentrations (43.3% and 54.4% inhibition for compounds **9** and **10** at 164 μM , compared to 72.7% for α -tocopherol at the same concentration). At low concentration no significant differences between the peroxidation-inhibiting activity of hydroquinone and quinone compounds were discernible. The results from these

Table 8. Cytotoxic Effects of Compounds **1**, **2**, **11**, **12**, and **14** toward the Cell Lines HM02, HepG2, and MCF7

	HM02			HepG2			MCF7		
	GI ₅₀ ^a	TGI ^b	LC ₅₀ ^c	GI ₅₀	TGI	LC ₅₀	GI ₅₀	TGI	LC ₅₀
1	2.3 ^d	7.4	>10	7.1	>10 ^e	>10	2.2	5.1	>10
2	1.8	6.0	>10	6.8	>10 ^f	>10	1.8	4.0	>10
11	0.3	0.6	3.4	1.8	4.0	>10	1.3	2.1	3.7
12	0.9	2.0	4.6	1.7	5.0	>10	0.9	1.7	3.6
14	0.9	2.4	4.8	1.8	3.5	8.4	1.6	2.5	4.0

^a GI₅₀ = 50% cell growth inhibition. ^b TGI = total cell growth inhibition. ^c LC₅₀ = 50% lethal concentration. ^d Concentrations in μg/mL. ^e 86% cell growth inhibition at 10 μg/mL. ^f 87% cell growth inhibition at 10 μg/mL.

antioxidant tests showed that the meroterpenoids obtained from *C. crinita* have powerful antioxidant activity.

The cytotoxic effects of compounds **1**, **2**, **11**, **12**, and **14** toward the cell lines HM02, HepG2, and MCF7 were investigated, and all compounds were found to be moderately active (Table 8). Since cytotoxicities for the isomeric pairs **1** and **2**, and **11** and **12**, were very similar, it seems that the *E/Z* geometry of the Δ⁶ double bond has little or no influence on the cytotoxic activity of these compounds. Additionally, the cytotoxicity profile of the five compounds tested was similar. Therefore, it can be presumed that the hydroquinone moiety in these molecules is the moiety responsible for this activity rather than the prenyl side chain part of the molecules.

Antibiotic activities of compounds **1–7** and **10–14** were tested in agar diffusion assays against the bacteria *Bacillus megaterium* and *Escherichia coli*, the fungi *Microbotrium violaceum*, *Eurotium repens*, and *Mycotypha microspora*, and the green microalga *Chlorella fusca*. In these assays all of the compounds were found to be devoid of activity. Compounds **1** and **2** were also tested for their ability to inhibit the enzyme HIV-1 reverse transcriptase, but were found to be inactive.

Experimental Section

General Experimental Procedures. Optical rotations were measured using a Jasco DIP 140 polarimeter equipped with an 1 mL cell, cell length 10.000 cm. UV and IR spectra were obtained employing Perkin-Elmer Lambda 40 and Perkin-Elmer Spectrum BX instruments, respectively. All NMR spectra were recorded on a Bruker Avance 300 DPX or 500 DRX spectrometer in *d*₆-acetone. Spectra were referenced to solvent signals with resonances at δ_{H/C} 2.04/29.8 (*d*₆-acetone). HREIMS were recorded on a Kratos MS 50 or a Finnigan MAT 95 spectrometer. HPLC was carried out using a Waters system consisting of a 600 pump, a 996 photodiode array detector, and a 717 plus auto sampler.

Plant Material. *Cystoseira crinita* Duby was collected in 2001 from the south coast of Sardinia, at depths of 0–3 m, and stored in EtOH at –4 °C until workup. The alga was identified by Dr. A. Flores-Moya, University of Málaga, Spain. A voucher species (voucher number MGC Phyc 3809) is deposited at the Herbarium of the Department of Plant Biology, Faculty of Sciences, University of Málaga, Spain.

Extraction and Isolation. After removal of the preservation EtOH, the alga was extracted with MeOH (3 × 1.0 L), followed by CH₂Cl₂ (3 × 1.0 L). Dry weight after extraction was 205.8 g. The CH₂Cl₂ extract was evaporated to dryness to yield 2.8 g of a dark brown extract. This material was fractionated by vacuum liquid chromatography (VLC) over silica gel (Si 60, 45–60 μm, Merck) using step-gradient elution from petroleum ether (PE, 100%) to MeOH (100%), to yield 10 fractions, each of 200 mL. ¹H NMR investigations of these fractions indicated VLC fraction 6 to be of further interest. Normal-phase silica HPLC (Knauer Eurospher-100 Si column, 250 × 8 mm, 5 μm, PE–acetone gradient elution from 80:20

to 65:35 in 20 min, 1.5 mL/min) and finally reversed-phase HPLC (Phenomenex RP-12 column, 250 × 4.6 mm, 5 μm, MeOH–H₂O (7:3), 1.0 mL/min) separation of this fraction yielded compounds **1** and **2**.

The MeOH and preservation EtOH extracts were combined and extracted with acetone (3 × 225 mL) to yield 3.1 g of a dark brown gum. This material was fractionated by VLC over silica gel (Si 60, 45–60 μm, Merck) using step-gradient elution from PE–EtOAc (60:40) to MeOH (100%), to yield seven fractions, each of 200 mL. The ¹H NMR spectrum of fraction 2 contained peaks characteristic for compounds like **1** and **2**. Further separation of this fraction by RP HPLC (Knauer Eurospher-100 RP-18 column, 250 × 4.6 mm, 5 μm, MeOH–H₂O (7:3), 1.0 mL/min) gave compounds **1**, **2**, **11**, and **12**, as well as three subfractions. Separation of subfraction 2.2 by NP HPLC (Knauer Eurospher-100 Si column, 250 × 8 mm, 5 μm, PE–EtOAc (77:23), 2 mL/min) yielded compounds **3**, **4**, and **13**. VLC fraction 1 was further fractionated by VLC (Si 60, 45–60 μm, Merck) employing step-gradient elution from PE to EtOAc to yield 11 subfractions, each of 100 mL. Of these, subfractions 7 and 8 showed characteristic tetraprenyltoluquinone signals in their ¹H NMR spectra. Subfraction 7 was separated by solid-phase extraction (Bakerbond SPE silica gel) with 9:1 PE–EtOAc as eluent followed by HPLC (Knauer Eurospher-100 Si column, 250 × 8 mm, 5 μm, PE–EtOAc (77:23), 2 mL/min) to give compounds **5** and **6**. Subfraction 8 was purified by HPLC (Knauer Eurospher-100 Si column, 250 × 8 mm, 5 μm, PE–EtOAc (83:17), 2 mL/min) and yielded compounds **7**, **8**, and **10**. The known compound **14** was obtained after VLC (Si 60, 45–60 μm, Merck) of original VLC fraction 3 using step-gradient elution from PE–EtOAc (8:2) to MeOH to give 10 fractions, each of about 50 mL, with fraction 4 being further purified by HPLC (Knauer Eurospher-100 Si column, 250 × 8 mm, 5 μm, PE–EtOAc (65:35), 2 mL/min).

2-[(2'E,6'E,10'E,14'Z)-5'-Oxo-15'-hydroxymethyl-3',7',11'-trimethylhexadeca-2',6',10',14'-tetraenyl]-6-methylhydroquinone (1): brown oil (51.1 mg, 0.025%); UV (EtOH) λ_{max} 236 nm (ε = 12 100), 290 nm (ε = 3400); IR (film) ν_{max} 3346, 2919, 1675, 1608, 1469 cm⁻¹; ¹H and ¹³C NMR data (see Tables 1 and 3); EIMS *m/z* (rel int) 426 (30), 424 (12), 408 (9), 255 (19), 192 (59), 177 (100), 175 (24), 137 (22), 135 (20), 121 (16), 107 (37), 95 (20), 93 (31), 81 (30); HREIMS *m/z* 426.2778 (calcd for C₂₇H₃₈O₄ 426.2770).

2-[(2'E,6'Z,10'E,14'Z)-5'-Oxo-15'-hydroxymethyl-3',7',11'-trimethylhexadeca-2',6',10',14'-tetraenyl]-6-methylhydroquinone (2): brown oil (30.3 mg, 0.015%); UV (EtOH) λ_{max} 236 nm (ε = 13 000), 285 nm (ε = 4200); IR (film) ν_{max} 3330, 2919, 1674, 1607, 1470 cm⁻¹; ¹H and ¹³C NMR data (see Tables 1 and 3); EIMS *m/z* (rel int) 426 (23), 424 (10), 408 (8), 255 (9), 192 (33), 177 (100), 175 (18), 137 (22), 121 (12), 107 (28), 95 (12), 93 (19), 81 (17); HREIMS *m/z* 426.2770 (calcd for C₂₇H₃₈O₄ 426.2770).

2-[(2'E,6'E,10'E)-5'-Oxo-13'-hydroxy-3',7',11',15'-tetramethylhexadeca-2',6',10',14'-tetraenyl]-6-methylhydroquinone (3): brown oil (1.2 mg, 0.001%); [α]_D²⁵ +4.6° (c 0.1; EtOH); UV (EtOH) λ_{max} 240 nm (ε = 11 300), 289 nm (ε = 2900); IR (film) ν_{max} 3344, 2920, 2854, 1677, 1654, 1609, 1470, 1438 cm⁻¹; ¹H and ¹³C NMR data (see Tables 1 and 3); EIMS *m/z* (rel int) 426 (6), 424 (11), 410 (9), 409 (19), 408 (62), 390 (6), 342 (12), 337 (17), 274 (11), 255 (20), 217 (12), 192 (21), 178 (13), 177 (100), 176 (17), 175 (56), 161 (10), 137 (24), 135 (46), 109 (17), 107 (24), 93 (23), 85 (26); HREIMS *m/z* 408.2669 (calcd for C₂₇H₃₆O₃ 408.2664).

2-[(2'E,6'Z,10'E)-5'-Oxo-13'-hydroxy-3',7',11',15'-tetramethylhexadeca-2',6',10',14'-tetraenyl]-6-methylhydroquinone (4): brown oil (1.4 mg, 0.001%); [α]_D²⁵ –5.4° (c 0.1; EtOH); UV (EtOH) λ_{max} 247 nm (ε = 13 900), 286 nm (ε = 3400); IR (film) ν_{max} 3345, 2921, 2853, 1676, 1654, 1610, 1439 cm⁻¹; ¹H and ¹³C NMR data (see Tables 1 and 3); EIMS *m/z* (rel int) 426 (1), 424 (2), 409 (7), 408 (23), 390 (25), 342 (4), 274 (9), 256 (7), 217 (7), 199 (7), 192 (22), 178 (13), 177 (100), 176 (16), 175 (87), 161 (9), 137 (25), 135 (24), 121 (13), 107 (31), 93 (15), 85 (20); HREIMS *m/z* 408.2668 (calcd for C₂₇H₃₆O₃ 408.2664).

2-[(2'E,6'E,10'E)-5'-Oxo-3',7',11',15'-tetramethylhexadeca-2',6',10',14'-tetraenyl]-6-methylhydroquinone (5): brown

oil (2.9 mg, 0.002%); UV (EtOH) λ_{\max} 243 nm ($\epsilon = 12\ 100$), 288 nm ($\epsilon = 3500$); IR (film) ν_{\max} 3400, 2923, 1682, 1654, 1610, 1472 cm^{-1} ; ^1H and ^{13}C NMR data (see Tables 1 and 3); EIMS m/z (rel int) 412 (6), 411 (28), 410 (100), 408 (9), 392 (6), 342 (20), 255 (39), 219 (40), 192 (17), 191 (20), 190 (28), 177 (59), 176 (23), 175 (54), 161 (11), 151 (25), 149 (15), 137 (74), 123 (17), 121 (13), 109 (24), 107 (12), 95 (14), 81 (44); HREIMS m/z 410.2822 (calcd for $\text{C}_{27}\text{H}_{38}\text{O}_3$ 410.2821).

2-[(2'E,6'Z,10'E)-5'-Oxo-3',7',11',15'-tetramethylhexadeca-2',6',10',14'-tetraenyl]-6-methylhydroquinone (6): brown oil (1.5 mg, 0.001%); UV (EtOH) λ_{\max} 236 nm ($\epsilon = 18\ 400$), 288 nm ($\epsilon = 5400$); IR (film) ν_{\max} 3400, 2922, 1683, 1654, 1610, 1456 cm^{-1} ; ^1H and ^{13}C NMR data (see Tables 1 and 3); EIMS m/z (rel int) 412 (7), 411 (31), 410 (100), 408 (11), 392 (7), 342 (7), 323 (17), 293 (7), 279 (7), 255 (27), 219 (32), 192 (39), 191 (20), 190 (31), 177 (85), 176 (25), 175 (74), 163 (22), 161 (13), 151 (55), 149 (29), 137 (41), 123 (18), 121 (15), 109 (62), 107 (14), 95 (15), 81 (32); HREIMS m/z 410.2821 (calcd for $\text{C}_{27}\text{H}_{38}\text{O}_3$ 410.2821).

2-[(2'E,6'E)-5'-Oxo-3',7',11'-trimethyldodeca-2',6',10'-tri-enyl]-6-methylhydroquinone (7): brown oil (1.9 mg, 0.001%); UV (EtOH) λ_{\max} 244 nm ($\epsilon = 9600$), 289 nm ($\epsilon = 2400$); IR (film) ν_{\max} 3391, 2923, 2851, 1676, 1653, 1610, 1472 cm^{-1} ; ^1H and ^{13}C NMR data (see Tables 1 and 3); EIMS m/z (rel int) 342 (14), 340 (6), 324 (2), 279 (4), 272 (2), 255 (6), 192 (6), 191 (11), 190 (55), 177 (6), 176 (9), 175 (45), 167 (6), 161 (4), 152 (11), 151 (100), 149 (19), 137 (9), 123 (27), 109 (18), 107 (9), 95 (13), 92 (6), 81 (8), 69 (81); HREIMS m/z 342.2200 (calcd for $\text{C}_{22}\text{H}_{30}\text{O}_3$ 342.2191).

2-[(2'E,6'Z)-5'-Oxo-3',7',11'-trimethyldodeca-2',6',10'-tri-enyl]-6-methylhydroquinone (8): brown oil (1.0 mg, 0.001%); UV (EtOH) λ_{\max} 244 nm ($\epsilon = 25\ 100$), 286 nm ($\epsilon = 8600$); IR (film) ν_{\max} 3376, 2919, 2854, 1674, 1652, 1608, 1466, 1440 cm^{-1} ; ^1H and ^{13}C NMR data (see Tables 1 and 3); EIMS m/z (rel int) 342 (100), 340 (7), 324 (10), 314 (3), 281 (3), 255 (19), 192 (43), 191 (13), 190 (17), 177 (33), 176 (13), 175 (43), 161 (10), 152 (10), 151 (86), 149 (8), 137 (31), 123 (34), 109 (33), 107 (13), 95 (17), 81 (11), 69 (32); HREIMS m/z 342.2200 (calcd for $\text{C}_{22}\text{H}_{30}\text{O}_3$ 342.2191).

5-Oxo-cystofuranoquinone (9): brown oil (0.7 mg, 0.0005%); UV (EtOH) λ_{\max} 250 nm ($\epsilon = 25\ 500$); IR (film) ν_{\max} 2923, 2854, 1684, 1654, 1614, 1437 cm^{-1} ; ^1H and ^{13}C NMR data (see Tables 2 and 4); EIMS m/z (rel int) 422 (15), 420 (21), 404 (2), 402 (2), 342 (7), 340 (8), 338 (13), 271 (6), 255 (9), 232 (11), 231 (67), 213 (11), 190 (57), 177 (19), 176 (16), 175 (48), 174 (22), 161 (9), 151 (43), 149 (100), 148 (32), 137 (13), 135 (13), 131 (24), 123 (13), 109 (16), 95 (27), 91 (17), 69 (33); HREIMS m/z 420.2306 (calcd for $\text{C}_{27}\text{H}_{32}\text{O}_4$ 420.2301).

5-Oxo-isocystofuranoquinone (10): brown oil (2.1 mg, 0.001%); UV (EtOH) λ_{\max} 250 nm ($\epsilon = 9400$); IR (film) ν_{\max} 2923, 2852, 1684, 1654, 1612, 1438 cm^{-1} ; ^1H and ^{13}C NMR data (see Tables 2 and 4); EIMS m/z (rel int) 422 (14), 420 (20), 404 (5), 342 (4), 271 (4), 255 (10), 232 (10), 231 (55), 192 (10), 191 (10), 190 (31), 177 (25), 176 (18), 175 (100), 161 (10), 151 (19), 149 (60), 137 (20), 135 (22), 131 (14), 123 (13), 121 (15), 109 (15), 107 (14), 105 (12), 95 (27), 91 (18); HREIMS m/z 420.2300 (calcd for $\text{C}_{27}\text{H}_{32}\text{O}_4$ 420.2301).

5-Oxo-cystofuranoquinol (11): brown oil (8.2 mg, 0.004%); spectroscopic data were consistent with the literature.¹¹

5-Oxo-isocystofuranoquinol (12): brown oil (6.1 mg, 0.003%); spectroscopic data were consistent with the literature.¹¹

2-[(2'E,6'E,10'E)-5',13'-Dioxo-3',7',11',15'-tetramethylhexadeca-2',6',10',14'-tetraenyl]-6-methylhydroquinone (13): brown oil (1.4 mg, 0.001%); spectroscopic data were consistent with the literature.¹¹

2-[(2'E,6'E,10'E,14'Z)-5'-Hydroxy-15'-hydroxymethyl-3',7',11'-trimethylhexadeca-2',6',10',14'-tetraenyl]-6-methylhydroquinone (14): brown oil (13.8 mg, 0.007%); $[\alpha]_{\text{D}}^{20} +1.0^\circ$ (c 1.28; EtOH); spectroscopic data were consistent with the literature.¹¹

Antioxidative Activity. Thiobarbituric Acid Reactive Substances Method (TBARS Assay). The assay was modified after Wallin et al.¹² and performed as previously described.¹³ Briefly, linolenic acid methyl ester was oxidized in

50 mM phosphate buffer (pH = 7.2), under FeSO_4 catalysis at 50 °C. Butylated hydroxytoluene (BHT) in ethanol was added to prevent further oxidation. Thiobarbituric acid reactive substances (TBARS) were determined using trichloroacetic acid and thiobarbituric acid at 60 °C for 30 min. The absorbance was read at 532 nm less the background absorbance at 600 nm.

Calculations. The percentage of inhibition was calculated from the absorbance readings and is expressed as the inhibition of lipid peroxidation of that sample compared to the not inhibited control (eq 1). A_{blank} = absorbance of the blank ($A_{532\text{ nm}} - A_{600\text{ nm}}$), A_{control} = absorbance of the control ($A_{532\text{ nm}} - A_{600\text{ nm}}$), A_{sample} = absorbance of the sample ($A_{532\text{ nm}} - A_{600\text{ nm}}$), and $A_{\text{sample blank}}$ = absorbance of the sample blank ($A_{532\text{ nm}} - A_{600\text{ nm}}$).

$$\% \text{ inhibition} = 100 - \frac{(A_{\text{sample}} - A_{\text{sample blank}}) \times 100}{A_{\text{control}} - A_{\text{blank}}} \quad (1)$$

α,α -Diphenyl- β -picrylhydrazyl (DPPH) Radical-Scavenging Effects. Assays were performed in flat bottom polystyrene 96-well microtiter plates. The DPPH radical-scavenging effects were measured using a modified previously established methodology.^{14,15} To 100 μL of each sample at different concentrations in EtOH were added 25 μL of DPPH (1 mM) in EtOH and 75 μL of EtOH. The resultant mixture was briefly shaken and maintained at room temperature in the dark for 30 min. At the end of this period the absorbance of the mixture was measured at 517 nm, using a SLT Spectral Rainbow microtiter plate reader.

Calculations. The percentage of scavenging of DPPH radical from a sample at a given concentration can be calculated from the absorbance readings as shown in eq 2.

$$\% \text{ scavenging} = 100 - \frac{(A_{\text{sample}} - A_{\text{sample blank}}) \times 100}{A_{\text{control}} - A_{\text{blank}}} \quad (2)$$

Trolox Equivalent Antioxidant Capacity (TEAC) Assay. The method used was adapted from Miller et al.¹⁶ and modified as described recently by Böhm et al.¹⁷ Briefly, the ABTS⁺ (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical cation was prepared by filtering a solution of ABTS (in phosphate-buffered saline (PBS)) through manganese dioxide powder. Excess manganese dioxide was removed from the filtrate by passing it through a 0.2 μm syringe filter. This solution was diluted in 5 mM PBS pH 7.4, adjusted to an absorbance of 0.700 at 734 nm and preincubated at room temperature prior to use for 2 h. The ABTS⁺ solution (1 mL) and 200 μL of the solution of antioxidants (diluted with ethanol) were vortexed for 30 s in reaction tubes, which were then centrifuged for 60 s at 10000 rpm. The absorbance (734 nm) of the lower phase (phase separation is achieved only with organic solutions of antioxidants) was taken exactly 2 min after initiation of mixing. PBS and ethanol blanks were run in each assay. The antioxidant activity of compounds was calculated by determining the decrease in absorbance according to the following equation:

$$\% \text{ antioxidant activity} = \left(\frac{A(\text{ABTS}^+) - A(\text{standard})}{A(\text{ABTS}^+)} \right) \times 100 \quad (A = \text{absorbance})$$

Different concentrations of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were used as standard.

Photochemiluminescence (PCL) Assay. In the PCL assay the photochemical generation of free radicals is combined with their sensitive detection by chemiluminescence. This reaction is induced by optical excitation of a photosensitizer S, which results in the generation of the superoxide radical $\text{O}_2^{\cdot-}$ as shown below:



The free radicals are visualized with a chemiluminescent detection reagent. Luminol works as photosensitizer as well as oxygen radical detection reagent. This reaction takes place

in the Photochem. The compounds were measured with the ACL kit.¹⁸ A 2.29 mL portion of reagent 1 (solvent and dilution reagent), 200 μ L of reagent 2 (buffer solution), 25 μ L of reagent 3 (photosensitizer), and 10 μ L of standard (Trolox in water) or sample (compounds in ethanol) solution were mixed and measured. The antioxidant potential was determined by using the integral under the curve.

Agar Diffusion Assay. Agar diffusion assays using the bacteria *Bacillus megaterium* and *Escherichia coli*, the fungi *Microbotrium violaceum*, *Eurotium repens*, and *Mycotypha microspora*, and the green microalga *Chlorella fusca* were done as described by Schulz et al. (1995).¹⁹ The tested concentration was 50 μ g/disk.

Cytotoxicity Tests. Cytotoxicity tests employing the cell lines HM02 (gastric adenocarcinoma), HepG2 (hepatocellular carcinoma), and MCF7 (breast adenocarcinoma) followed the standards of the NCI.²⁰

HIV-1-Reverse Transcriptase (RT) Assay. The inhibition of HIV-1-RT activity was measured according to the ELISA protocol established by Eberle and Seibl.²¹ The tested concentration of **1** and **2** was 66 μ g/mL.

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