Phytochemistry, Vol 27, No 5, pp 1327 1331, 1988
Printed in Great Britain

0031 9422/88 $3 00 + 0 00
Pergamon Press plc

ANTIMICROBIAL TETRAPRENYLTOLUQUINOL DERIVATIVES FROM *CYSTOSEIRA SPINOSA* VAR. *SQUARROSA*

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(*Received* 10 *July* 1987)

Key Word Index—*Cystoseira spinosa* var *squarrosa*, Cystoseiraceae, brown algae, cystofuranoquinols, tetraprenyltoluquinols

Abstract—Six new tetraprenyltoluquinol derivatives have been isolated from the brown alga *Cystoseira spinosa* var. *squarrosa* and their structures determined by chemical and spectral methods. Three of these compounds possess antimicrobial activity against both gram-positive and gram-negative bacteria

INTRODUCTION

In the course of our continuing investigation of Mediterranean algae for the presence of biologically active metabolites, we observed that the crude dichloromethane extract of the brown seaweed *Cystoseira spinosa* var *squarrosa* possesses antimicrobial activity against both gram-positive and gram-negative bacteria Since preliminary experiments indicated that most of the activity was associated with fractions containing tetraprenyltoluquinol derivatives, these were examined in detail and six novel compounds (**1**–**6**) were isolated.

RESULTS AND DISCUSSION

The over-all spectral data strongly suggested that the six new compounds isolated from *C spinosa* var. *squarrosa* together with the known geranylgeranyltoluquinone were closely related to the previously reported *Cystoseira* tetraprenyltoluquinols [1]

The least polar of the new metabolites, 5-oxo-*iso*cystofuranoquinol* (**1**), $C_{27}H_{34}O_4$ (HREIMS), was obtained as an optically inactive oil. The UV spectrum was consistent with the presence of a hydroquinol [λ_{max} 287 (ε=4800) and 218 (ε=30 000) nm] and an enone [λ_{max} 246 (ε=14 600) nm] chromophore The presence of a conjugated carbonyl was also evident from the appropriate resonance (δ198 6, *s*) in the ^{13}C NMR spectrum and the relevant band (1676 cm^{-1}) in the IR spectrum, which also contained strong olefinic bands at 1660 and 1610 cm^{-1}, and absorptions at 1550 and 900 cm^{-1} indicative of a furan ring The presence of this heterocycle was confirmed by the ^{1}H NMR data, which in addition indicated a 2,4-substitution pattern (δ7.06, H-16′; 5.88, H-14′, 3.21, H-12′; 1.98, H-17′) Pertinent resonances for the furan ring (154.4, *s*, C-13′; 108.8, *d*, C-14′, 120.4, *s*, C-15′, 137.7, *d*, C-16′) and the carbons directly bonded to it (38 4, *t*, C-12′; 9.7, *q*, C-17′) were present in the ^{13}C NMR spectrum (Table 1) thus allowing us to define the structure of the terminal isoprene unit.

The ^{1}H NMR also included (Table 2) an AB system (δ6.52 and 6.48) assignable to two *meta*-coupled aromatic protons, and signals for a methyl on a benzene ring (δ2.20) and a benzylic methylene (δ3 33, J=7.5 Hz, H-1′) coupled with the olefinic proton at δ5 37 (J=7 5 Hz, H-2′). The last was in turn long-range coupled with both the vinyl methyl at δ1 74 and the methylene at δ3 14 (H-4′). These data clarified the structure of the aromatic moiety and the vicinal isoprene unit, which was confirmed by the presence in the MS spectrum of an intense fragment at *m/z* 175, assignable to the stabilized oxonium ion A The low-field position of the protons at C-4′ revealed the proximity to the conjugated carbonyl function, thus allowing us to extend the part structure to include a second isoprene residue Assignment of the rest of the signals in the ^{1}H NMR spectrum was trivial and led to structure **1** for the novel algal metabolite The proposed structure agreed with the ^{13}C NMR data, which also allowed assignment of the *Z* geometry to the C-6′ double bond based on the diagnostic values of the chemicl shifts of C-8′ (33 7 ppm) and C-19′ (25.6) [2].

The second compound (**2**) isolated as an optically inactive oil was an isomer of **1**. Its spectral properties [λ_{max} 220 (ε=24 000), 243 (ε=15 300) and 288 (ε=4700) nm, ν_{max} 3370, 1675, 1610, 1550 and 900 cm^{-1}] closely resembled those of **1**, and the mass spectra of the two compounds were almost superimposable. The ^{1}H NMR spectrum of **2** (Table 2), when compared with that of **1**, displayed differences confined to the region influenced by the stereochemistry of the C-6′ double bond; in fact, the chemical shift of the vinyl methyl at C-7′ moved downfield from δ1.89 in **1** to δ2 18 in **2** and concomitantly the C-8′ methylene shifted upfield from δ2 64 to *ca* δ2, indicating a change in the geometry of the double bond from *Z* in **1** to *E* in **2** (comparable differences are observed in the spectra of related couples of geometrical isomers [3]). Analogously, in the ^{13}C NMR spectrum of **2** (Table 1) C-8′ and C-9′ were the only carbon atoms whose chemical shift differed significantly from those of the corresponding atoms in **1**. Based on the

*We have named the parent compound of **1**, *iso*cystofuranoquinol (see formula) The hypothetical all-*trans* isomer has been named cystofuranoquinol

Table 1 ^{13}C NMR data of compounds **1–6** (75 MHz, $CDCl_3$, TMS as int standard)*

C	1	2	3	4	5	6
1	149 2 *s*	149 3 *s*	149 3 *s*	149 2 *s*	149 4 *s*	149 6 *s*
2	132 6 *s*[a]	132 9 *s*[a]	132 1 *s*	131 3 *s*	134 1 *s*	131 5 *s*[a]
3	114 1 *d*	114 0 *d*	114 2 *d*	114 2 *d*	114 0 *d*	114 1 *d*
4	146 4 *s*	145 9 *s*	146 1 *s*	146 2 *s*	146 0 *s*	145 8 *s*
5	115 6 *d*	115 5 *d*	115 5 *d*	115 5 *d*	115 5 *d*	115 5 *d*
6	127 7 *s*	127 7 *s*	127 9 *s*	127 8 *s*	127 9 *s*	127 7 *s*
1′	30 1 *t*	29 9 *t*	30 1 *t*	30 1 *t*	29 8 *t*	29 9 *t*
2′	126 8 *d*	127 1 *d*[b]	127 4 *d*[a]	127 3 *d*[a]	128 4 *d*[a]	127 2 *d*[b]
3′	126 0 *s*	126 1 *s*	125 7 *s*	125 7 *s*	125 7 *s*	127 1 *s*
4′	55 0 *t*	54 9 *t*	47 8 *t*	47 9 *t*	48 0 *t*	55 2 *t*[c]
5′	198 6 *s*	199 9 *s*	66 4 *d*	66 4 *d*	66 4 *d*	199 8 *s*
6′	123 2 *d*[b]	122 5 *d*[b]	126 1 *d*[a]	123 8 *d*[a]	124 3 *d*[a]	122 4 *d*[b]
7′	160 2 *s*	160 0 *s*	134 0 *s*[b]	134 3 *s*	134 3 *s*[b]	159 2 *s*
8′	33 7 *t*	41 0 *t*	39 2 *t*[c]	39 7 *t*[b]	39 7 *t*[c]	40 8 *t*
9′	26 8 *t*	25 8 *t*	26 3 *t*	26 8 *t*[c]	26 3 *t*[d]	26 1 *t*
10′	125 9 *d*[b]	125 1 *d*[b]	125 7 *d*[a]	124 4 *d*[a]	125 7 *d*[a]	127 8 *d*[b]
11′	132 1 *s*[a]	131 5 *s*[a]	138 5 *s*	138 9 *s*[d]	138 3 *s*[b]	130 4 *s*[a]
12′	38 4 *t*	38 2 *t*	38 4 *t*[c]	39 5 *t*[b]	39 3 *t*[c]	54 9 *t*[c]
13′	154 4 *s*	153 9 *s*	154 3 *s*	26 4 *t*[c]	26 0 *t*[d]	199 6 *s*
14′	108 8 *d*	108 8 *d*	108 8 *d*	125 6 *d*[a]	127 6 *d*[a]	123 0 *d*[b]
15′	120 4 *s*	120 4 *s*	120 4 *s*	135 4 *s*[d]	134 9 *s*[b]	156 0 *s*
16′	137 7 *d*	137 7 *d*	137 7 *d*	25 6 *q*	21 2 *q*	27 6 *q*
17′	9 7 *q*	9 6 *q*	9 7 *q*	17 6 *q*	61 6 *t*	20 7 *q*
18′	16 1 *q*[c]	16 4 *q*[c]	16 0 *q*[d]	16 0 *q*[c]	16 5 *q*[c]	16 6 *q*[d]
19′	25 6 *q*	19 6 *q*	16 4 *q*[d]	16 6 *q*[c]	16 1 *q*[c]	19 4 *q*
20′	16 7 *q*	16 8 *q*[c]	16 5 *q*[d]	16 4 *q*[c]	16 4 *q*[c]	16 6 *q*[d]
6-Me	15 8 *q*[c]	16 0 *q*[c]	15 9 *q*[d]	16 0 *q*[c]	16 1 *q*[c]	16 1 *q*[d]

*Multiplicities were obtained by off-resonance decoupling experiments

[a–e]Values with identical superscript within each column can be interchanged

above evidence, the structure of the new cystofuranoquinol was definitely assigned as **2**.

Compound **3** had a molecular formula $C_{27}H_{36}O_4$ (HREIMS) and was an optically active clear oil $[\alpha]_D = +1.2°$. The UV spectrum exhibited bands at 221 ($\varepsilon = 12\,500$) and 290 ($\varepsilon = 2800$) nm, while the IR spectrum showed absorptions for hydroxyl(s) and furan ring. Consideration of the ^{13}C and ^{1}H NMR data (Tables 1 and 2) suggested **3** to be the allylic alcohol corresponding to **2**, confirmation of this assumption came from sodium borohydride reduction of **2**, which afforded a compound indistinguishable from **3**, apart from the optical rotation ($[\alpha]_D = 0$). It is worth noting that in the ^{1}H NMR spectrum of **3** the signal of the benzylic methylene, which in the preceding compounds is an AB system, appears as the AB part of an ABX system, as a consequence of restricted rotation caused by intramolecular hydrogen bonding.

Compound **4** was isolated as a yellow oil, $[\alpha]_D = +1°$. Its elemental composition, established as $C_{27}H_{40}O_3$ by combustion analysis and ^{13}C NMR spectroscopy, required eight degrees of unsaturation, taking into account that the ^{13}C NMR spectrum of **4** contained resonances for eight olefinic carbons and assuming a tetraprenyltoluquinol structure, this indicated an uncyclized side chain. The EIMS of **4** did not show a molecular ion peak at *m/z* 412, but showed a peak at *m/z* 394 for the ion $[M - H_2O]^+$, moreover, an intense fragment at *m/z* 175 indicated the presence of a partial structure including the toluquinol moiety and the first isoprene unit. The appropriate signals in both ^{13}C and ^{1}H NMR spectra (Tables 1 and 2) supported this partial structure, while ^{1}H NMR decoupling experiments permitted the hydroxyl to be located at C-5′. In fact, the hydroxymethine proton at δ4.54 was coupled with both an olefinic proton and a methylene, the latter was positively located at C-4′ by its allylic coupling with a vinylic proton (δ5.37 *t*, $J = 7.2$ Hz, H-2′) in turn long range coupled with the vinylic methyl at C-20′. The remaining signals in the ^{13}C and ^{1}H NMR spectra allowed the new metabolite to be formulated as **4**, in which the all-*trans* geometry of the side chain was deduced from the chemical shifts of the pertinent methyls in the ^{13}C NMR spectrum.

Another metabolite, **5**, was assigned a molecular formula of $C_{27}H_{40}O_4$ on the basis of elemental analysis and ^{13}C NMR. The EIMS contained no molecular ion, the highest *m/z* ion being at *m/z* 410 $[M - H_2O]^+$. The structure of **5** was established from comparison of its spectral data with those of **4**, to which it was obviously related. From the ^{1}H NMR spectrum it was evident that compound **5** one of the six methyl groups present in **4** was replaced by a hydroxymethylene group (2H *br s* at δ4.10). This was confirmed by the ^{13}C NMR spectrum of **5**, which displayed a triplet at δ61.6 replacing a methyl quartet in **4**. In order to accommodate the displacement

Table 2. ^{1}H NMR data of compounds **1–6** (300 MHz, $CDCl_3$, TMS as int standard)*

H	1	2	3	4	5	6
3 5	6 52 6 48 } AB (3)	6 55 6 52 } AB (3)	6 48 6 44 } AB (3)	6 49 6 44 } AB (3)	6 49 6.44 } AB (3)	6 51 6 49 } AB (3)
1′	3 33 *d* (7 5)	3 33 *d* (7.5)	3 32 *dd* (15, 8) 3 22 *dd* (15, 7)	3.34 dd (15, 7 6) 3 24 *dd* (15, 6.8)	3 30 dd (15, 8) 3 20 *dd* (15, 7)	3 31 *d* (7 5)
2′	5 37 *t* (7.5)	5 35 *t* (7 5)	5.35 *t* (7 5)	5.37 *t* (7 2)	5.36 *t* (7 5)	5.39 *t* (7 5)
4′	3 14 *br s*	3.17 *br s*	2 20 *m*	2 22 *m*	2.23 *m*	3 10 *br s*
5′	—	—	4 54 *ddd* (8, 8, 5)	4 54 *ddd* (8, 8, 6)	4 51 *ddd* (8, 8, 6 5)	—
6′	6 10 *br s*	6 12 *br s*	5 19 *d* (8)	5 19 *d* (8)	5 14 *d* (8)	6 09 *br s*
8′	2.64 *t* (7)	2.0–2.2†	2.0–2.1†	1.9–2.1†	1.9–2.1†	2.1–2.2†
9′	2.0–2.2†					
10′	5 24 *t* (7 5)	5 20 *t* (6 5)	5 18 *t* (7)	5 10 *t* (7)	5 06 *t* (7)	5 18 *t* (7)
12′	3 21 *br s*	3 25 *br s*	3 23 *br s*	1.9–2 1†	1.9–2.1†	3 03 *br s*
13′	—	—	—			—
14′	5 88 *br s*	5.86 *br s*	5 88 *br s*	5 10 *t* (7)	5 26 *t* (7)	6.09 *br s*
16′	7 06 *br s*	7 07 *br s*	7 07 *br s*	1 60 *br s*	1.76 *br s*	1.86 *br s*
17′	1 98 *br s*	2 01 *br s*	2 00 *br s*	1 69 *br s*	4.10 *br s*	2 12 *br s*
18′	1.61 *br s*	1 63 *br s*	1 61 *br s*	1 60 *br s*	1.55 *br s*	1.57 *br s*
19′	1 89 *br s*	2 18 *br s*	1 71 *br s*	1 70 *br s*	1 64 *br s*	2 10 *br s*
20′	1 74 *br s*	1 77 *br s*	1 81 *br s*	1 79 *br s*	1 75 *br s*	1.70 *br s*
6-Me	2 20 *s*	2 22 *s*	2.15 *s*	2.16 *s*	2 15 *s*	2 15 *s*
OH	5.60 *br s*	6.63 *br s*	5 12 *br s*	5 79 *br s*	—	—
OH	5 05 *br s*	—	—	4 94	—	—

*Coupling constants (*J* in parentheses) are given in Hz
†Overlapped with other signals.

of the resonance of the 16′-Me (identified from the characteristically downfield position of its chemical shift) from δ25.6 in **4** to δ21 2 in **5**, the hydroxymethylene had to replace 17′-Me. Thus, for all the carbons of the terminal isoprenoid unit an excellent agreement was obtained with the literature values for related compounds [4]. Therefore, this metabolite was assigned structure **5**.

The last compound (**6**) had molecular formula $C_{27}H_{36}O_4$ (HREIMS) and was an optically inactive oil The presence of two conjugated carbonyls was evident from the IR (ν_{max} 1680 cm^{-1}) and ^{13}C NMR (199 6 and 199 8 ppm) spectra. The mass spectrum, in addition to a prominent peak at *m/z* 175 which indicated that the initial part of the molecule was the same as in all the compounds discussed above, contained a base peak at *m/z* 83 $[(Me)_2C{=}CH{-}C{\equiv}O]^+$ attributable to a terminal isoprenoid unit This and a comparison of the spectral data (Tables 1 and 2) with those of **2** led to structure **6** for the new metabolite.

Similarly to what is observed for the cystofuranoquinols, in the above open-chain compounds the benzylic methylene is seen as the AB part of an ABX system when an hydroxyl, but not a carbonyl group, is present at position 5

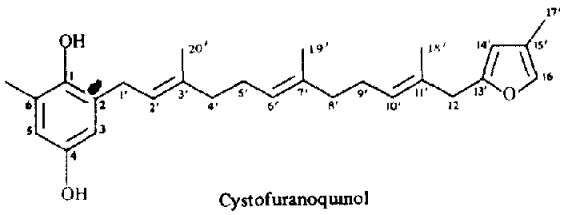
Each new metabolite was tested for antibiotic activity against gram-positive (*Staphylococcus aureus, Streptomyces faecalis, Micrococcus luteus, Bacillus subtilis*) and gram-negative organisms (*Aeromonas hydrophyla, Hafnia alvei, Proteus mirabilis, Escherichia coli*). Compound **3** was considerably active against all human pathogens used except *S. faecalis*, compound **5** moderately inhibited *S. aureus, A. hydrophyla* and *M. luteus*, and **1** exhibited moderate activity against *S aureus* The remaining compounds were inactive.

EXPERIMENTAL

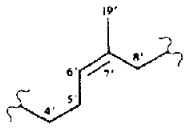
General experimental procedures ^{1}H and ^{13}C NMR 300 and 75 MHz respectively, $CDCl_3$, TMS as int stand Low resolution MS 70 eV, high-resolution MS Kratos MS-50S Final purification of all metabolites was achieved by prep LC on silica gel (LiChrosorb Si-60, 25–40 μ) using a Jobin-Yvon Miniprep liquid chromatograph TLC was carried out using glass-backed precoated silica gel F_{254} plates (Merck) Compounds were detected by spraying with 10% soln of $Ce(SO_4)_2$ in 1 M H_2SO_4, or by UV light (254 nm). All solvents were spectral grade or distilled prior to use

Plant material Cystoseira spinosa Sauv var *squarrosa* (De Notaris) was collected on rocks at about 3 m depth in June 1986 at Portopalo, near Pachino, Sicily A voucher specimen was deposited in the Herbarium of the Istituto e Orto Botanico, Catania, Italy.

Extraction and purification. Shade-dried and ground plant material (150 g) was extracted (× 3) with CH_2Cl_2 at room temp with continuous stirring. The combined extracts were evapd *in vacuo* to yield the final crude extract as a dark green oil (2.4 g) which was chromatographed on silica gel (100–200 mesh, 150 g) with hexane containing increasing amounts of Et_2O Fractions of 50 ml were collected and those exhibiting similar TLC profiles were combined Fractions 5 and 6 contained only the known 2′-geranylgeranyl-6′-methylbenzoquinone (23 mg, 0.015% dry wt) Fractions 25 and 26 contained predominantly **1** and **2** which were separated by prep LC using CH_2Cl_2 as eluant to give pure **1** (40 mg, 0.026% dry wt) and **2** (145 mg, 0 096% dry wt)



Cystofuranoquinol

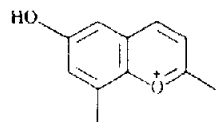


iso-Cystofuranoquinol

Fractions 27–31 were further purified by prep LC (Et_2O–C_6H_{14}, 5 6, followed by Me_2CO–$CHCl_3$, 1 32) to give **4** (32 mg, 0 021% dry wt) and **6** (52 mg, 0.034% dry wt) Finally, fractions 32 and 33 were purified by prep LC (Et_2O–C_6H_{14}, 1 1) to yield pure **3** (85 mg, 0 056% dry wt) and **5** (34 mg, 0 023% dry wt) All compounds were obtained as oils

5-Oxo-isocystofuranoquinol (**1**) IR ν_{max}^{film} cm^{-1} 3390, 2930, 1675, 1660, 1610, 1550, 1470, 900, UV λ_{max}^{EtOH} nm 218 (ε = 30 000), 246 (ε = 14 600), 287 (ε = 4800), HRMS $[M]^-$ 422 2458 (calc for $C_{27}H_{34}O_4$ 422 2456), MS *m/z* (rel int) 422 (29), 404 (12), 255 (15), 221 (59), 177 (82), 175 (72), 149 (base), 137 (67), 135 (35), 121 (41), 109 (29), 107 (29), 105 (29), 95 (56), 93 (35), 91 (47), 83 (18), 81 (21), 79 (29), 77 (26), 69 (18), 67 (23), 65 (22), 55 (18) 53 (18), 43 (18), 41 (29)

5-Oxo-cystofuranoquinol (**2**), IR ν_{max}^{film} cm^{-1} 3370, 2920, 1675, 1610, 1550, 1470, 900; UV λ_{max}^{EtOH} nm 220 (ε = 24 000), 243 (ε = 15 300), 288 (ε = 4700), HRMS $[M]^+$ 422 2452 (calc. for $C_{27}H_{34}O_4$ 422 2456), MS *m/z* (rel int) 422 (23), 404 (3), 255 (16), 221 (39), 177 (27), 175 (39), 149 (base), 137 (19), 135 (11), 131 (26), 121 (16), 109 (8), 107 (10), 105 (13), 95 (19), 93 (10), 91 (19), 83 (8),



A

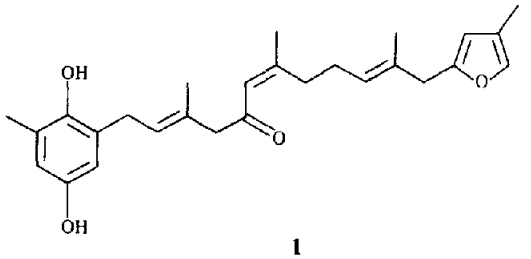
81 (13), 79 (13), 77 (10), 69 (19), 67 (6), 55 (11), 43 (16), 41 (16)

5-Hydroxy-cystofuranoquinol (**3**) $[\alpha]^{20}$ (c) +1 2 (589), +3 6 (578), +6 1 (546) (EtOH, c 2 8), IR ν_{max}^{film} cm^{-1} 3360, 2930, 1670, 1610, 1550, 1470 900, UV λ_{max}^{EtOH} nm 221 (ε = 12 500), 290 (ε = 2800), HRMS $[M]^+$ 424 2611 (calc for $C_{27}H_{36}O_4$ 424 2613), MS *m/z* (rel int) 424 (7), 406 (13), 257 (12), 225 (11), 192 (10), 177 (60), 175 (40), 149 (77), 137 (base), 123 (30), 121 (30), 95 (33), 91 (33), 83 (53), 55 (20), 43 (20), 41 (27)

2-[(2′E,6′E,10′E)-5′-hydroxy-3′,7′,11′,15′-tetramethylhexadeca-2′,6′,10′,14′-tetraenyl]-6-Methylhydroquinone (**4**) $[\alpha]^{20}$ (c) +1 0 (589), +1 1 (578), +1 2 (546) (EtOH, c 2 3), IR ν_{max}^{film} cm^{-1} 3380, 2920, 1660, 1610, 1470, UV λ_{max}^{EtOH} nm 220 (ε = 11 000), 290 (ε = 3500), HRMS $[M - H_2O]^+$ 394 2868 (calc for $C_{27}H_{38}O_2$ 394 2871), MS *m/z* (rel int) 394 (14), 288 (5), 257 (21), 192 (25), 177 (79), 175 (32), 150 (39), 137 (base), 121 (29) 109 (18), 95 (18), 81 (43) 69 (80), 55 (18) 43 (14), 41 (50) (Found C, 78 50, H, 9 62 $C_{27}H_{40}O_3$ requires C 78 64, H, 9 71)

2-[(2′E,6′E,10′E,14 Z)-5′-hydroxy-15′-hydroxymethyl-3′,7′,11′-trimethylhexadeca-2′,6′,10′,14′-tetraenyl]-6-Methylhydroquinone (**5**) IR ν_{max}^{film} cm^{-1} 3360, 2920, 1655, 1615, 1470, UV λ_{max}^{EtOH} nm 220 (ε = 13 700), 290 (ε = 4100), HRMS $[M - H_2O]^+$ 410 2827 (calc for $C_{27}H_{38}O_3$ 410 2820), MS *m/z* (rel int) 410 (7), 257 (13), 255 (11), 215 (8), 192 (17) 177 (86), 175 (71), 149 (21), 137 (base), 131 (37), 109 (20), 107 (36), 105 (17) 95 (27), 93 (37), 91 (27), 81 (36), 79 (24), 69 (26), 67 (26) 55 (37), 43 (57), 41 (36) (Found C, 75 65, H, 9 30 $C_{27}H_{40}O_4$ requires C, 75 70, H, 9 35)

2-[(2′E,6′E,10′E)-5′ 13′-dioxo-3′,7′ 11 ,15′-tetramethylhexadeca-2′,6′ 10′,14′-tetraenyl]-6-Methylhydroquinone (**6**) IR ν_{max}^{film} cm^{-1} 3390, 2920, 1680, 1625, 1470, UV λ_{max}^{EtOH} nm 240 (ε = 32 000) 285 (ε = 6600), HRMS $[M]^+$ 424 2610 (calc for



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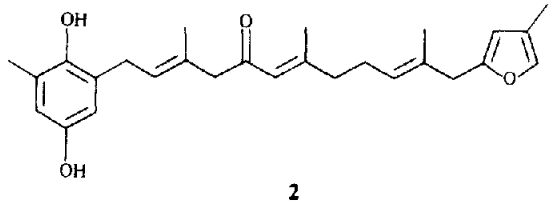

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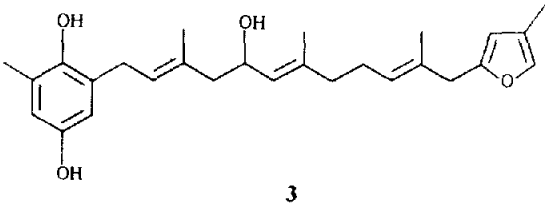

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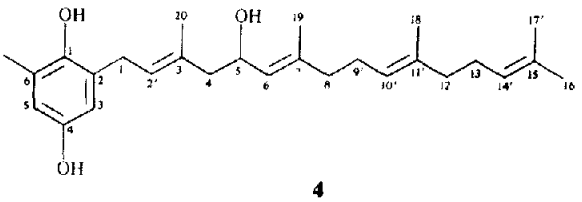

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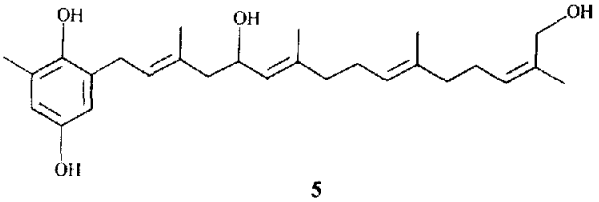
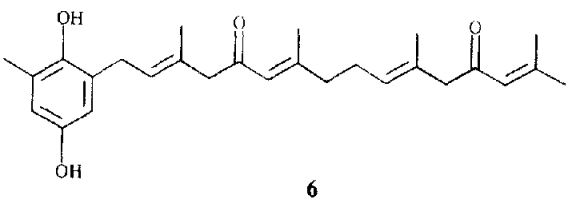


5



6

$C_{27}H_{36}O_4$ 424 2613), MS *m/z* (rel int): 424(14), 406 (2), 255 (12), 177 (37), 175 (25), 137 (30), 109 (10), 107 (11), 97 (17), 91 (10), 83 (base), 69 (12), 55 (30), 43 (9), 41 (12).

Reduction of **2** *to give* **3** $NaBH_4$ (3 mg) was added to a soln of **2** (10 mg) in EtOH (1 ml) and the mixture was stirred for 20 min After addition of H_2O the organic matter was extracted (×3) with Et_2O. Evapn of the solvent left a residue which was subjected to prep. LC (Et_2O–C_6H_{14}, 1:1) to give optically inactive **3** (5 mg), identified by comparison of the physical properties (IR, UV, NMR, MS) with those of the natural compound

Bioassays Antimicrobial assays were carried out using the standard agar plate-assay disc method at disc concentrations of 100 μg Zones of inhibition in excess of 4 mm were interpreted as positive inhibitions.

Acknowledgements—This work was financially supported by Ministero Pubblica Istruzione (Rome) Thanks are due to Prof. Giuseppe Giaccone, Istituto e Orto Botanico, University of Catania, for identification of plant material. The authors are grateful to Prof Guido Sodano, Facoltà di Scienze, University of Basilicata, for kind permission to use the Bruker AM-300 instrument in the present study We also wish to thank Dr. Corrada Geraci and Prof Salvatore Caccamese for antimicrobial assays

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