A Cytotoxic and Antifungal 1,4-Naphthoquinone and Related Compounds from a New Zealand Brown Alga, Landsburgia quercifolia

Nigel B. Perry, John W. Blunt, and Murray H. G. Munro

J. Nat. Prod., **1991**, 54 (4), 978-985• DOI:
10.1021/np50076a009 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 54, No. 4, pp. 978-985, Jul-Aug 1991

A CYTOTOXIC AND ANTIFUNGAL 1,4-NAPHTHOQUINONE AND RELATED COMPOUNDS FROM A NEW ZEALAND BROWN ALGA, *LANDSBURGIA QUERCIFOLIA*

NIGEL B. PERRY,[1] JOHN W. BLUNT,* and MURRAY H.G. MUNRO*

Department of Chemistry, University of Canterbury, Christchurch, New Zealand

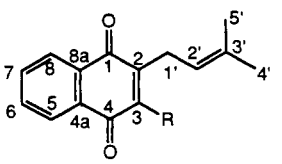
ABSTRACT.—The bioactivity-directed isolation of deoxylapachol [**1**] from a New Zealand brown alga, *Landsburgia quercifolia*, is described. Compound **1** was active against P-388 leukemia cells (IC_{50} 0.6 μg/ml) and was also antifungal. 1,4-Dimethoxy-2-(3-methyl-2-butenyl)-naphthalene [**3**] was the major low polarity component of extracts of this seaweed, which also contained 2,3-dihydro-2,2-bis(3-methyl-2-butenyl)-1,4-naphthalenedione [**6**] and 2-(3-methyl-2-butenyl)-2,3-epoxy-1,4-naphthalenedione 4,4-dimethoxy ketal [**7**]. Compound **7** was converted to the 2,3-epoxide of **1**, which had biological activities similar to those of **1**.

More than 60% of the species of brown algae (phylum Phaeophyta) collected from around New Zealand gave extracts which were cytotoxic toward P-388 leukemia cells (1). We now report that deoxylapachol [**1**] is the major cytotoxic component of one of these species, *Landsburgia quercifolia* (Hook. fil. et Harv.) Harv. (family Cystoseiraceae, order Fucales). Various related compounds were isolated and identified, including 1,4-dimethoxy-2-(3-methyl-2-butenyl)-naphthalene [**3**], the major low polarity component of extracts of this seaweed from three different locations.

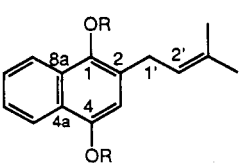
Landsburgia is a genus endemic to New Zealand with two distinct species: *L. quercifolia*, which grows on the North and South Islands, and *Landsburgia myricaefolia*, which is endemic to the Chatham Islands (2). The only previous report on chemical components of either species described the carotenoid content of *L. quercifolia* (3). A recent review found two reports of low-molecular-weight cytotoxic compounds from other genera in the family Cystoseiraceae, both diterpene-quinol compounds (4).

RESULTS

BIOACTIVITY-DIRECTED ISOLATION OF DEOXYLAPACHOL [**1**].—Preliminary chromatographic studies on a small-scale extract of *L. quercifolia* showed good recovery of the bioactivity in low-polarity fractions from both octadecyl-coated reversed-phase (C_{18} rp) and Si gel normal phase columns. Consequently a larger-scale extract was partitioned between H_2O and CH_2Cl_2, which concentrated the bioactivity in the organic phase. One subsample of the combined active fractions from reversed-phase flash chromatography was subjected to C_{18} rplc at higher resolution, while another subsample was fractionated by normal phase Si gel tlc. Assays of the fractions from both of these



1 R=H
2 R=OH



3 R=Me
4 R=H

[1]Present address: Plant Extract Research Unit, Department of Chemistry, University of Otago, P.O. Box 56, Dunedin, New Zealand.

TABLE 1. ^{1}H- and ^{13}C-nmr Data for Naphthoquinone Derivatives from *Landsburgia quercifolia*[a]

Position	Compound								
	1		**3**		**6**		**7**		**8**
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	—	185.38[b]	—	151.80[b]	—	200.36[b]	—	194.60	—
2	—	150.84	—	129.48[c]	—	54.11	—	60.35	—
3	6.76 (t, 1.7)	134.66	6.60	105.47	2.91	47.02	3.86	57.20	3.84
4	—	185.35[b]	—	146.53[b]	—	196.80[b]	—	98.40	—
4a	—	132.17[c]	—	125.33[c]	—	132.25[c]	—	136.13[b]	—
5	8.02–8.12 (m)[b]	126.55[d]	8.18 (8.3, 1.5, 0.8)	122.21	8.07 (m)[b]	127.27[d]	7.68 (8.0, 1.5)	127.01	8.02 (m)[b]
6	7.68–7.75 (m)[b]	133.59[e]	7.41 (8.2, 6.8, 1.3)	124.64	7.66–7.75 (m)[b]	133.82[e]	7.60 (7.5, 7.5, 1.5)	132.75	7.70–7.77 (m)[b]
7	7.68–7.75 (m)[b]	133.64[e]	7.50 (8.2, 6.9, 1.4)	126.39	7.66–7.75 (m)[b]	134.09[e]	7.47 (7.5, 7.5, 1.5)	129.41	7.70–7.77 (m)[b]
8	8.02–8.12 (m)[b]	126.07[d]	8.02 (8.3, 1.4, 0.8)	121.64	8.00 (m)[b]	125.73[d]	7.90 (7.8, 1.5)	127.69	7.95 (m)[b]
8a	—	136.42[c]	—	128.64[c]	—	135.94[c]	—	130.19	—
1′	3.26 (br d, 7.3)	28.03	3.51 (dm, 7.1, 1.1)	28.65	2.44 (14.4, 8.4) 2.23 (14.4, 6.8)	36.11	2.88 (15.6, 7.8) 2.70 (15.6, 6.7)	26.36	3.04 (15.5, 8.2) 2.69 (15.4, 6.8)
2′	5.21 (tm, 7.3, 1.4)	118.20	5.34 (tm, 7.1, 1.4)	123.07	5.00 (tm, 7.5, 1.5)	118.91	5.13 (tm, 7.3, 1.4)	116.43	5.10 (tm, 7.6, 1.4)
3′	—	132.36[c]	—	132.54	—	134.63[c]	—	136.19[b]	—
4′	1.77 (q, 1.2)	25.78	1.75 (q, 1.3)	25.63	1.60 (br d, 1.1)	25.87	1.73 (q, 1.3)	25.83	1.72 (q, 1.4)
5′	1.66 (br d, 1.2)	17.81	1.80 (br d, 1.0)	17.95	1.50 (br d, 1.3)	17.88	1.69 (br d, 0.9)	18.09	1.68 (br d, 1.0)
1-OMe	—	—	3.86	61.91	—	—	—	—	—
4-OMe	—	—	3.96	55.54	—	—	3.61 3.02	50.26 49.60	—

[a]In $CDCl_3$; shifts in ppm (couplings in Hz (±0.2); observed multiplicities: d=doublet, t=triplet, q=quartet, m=multiplet, br=broad).
[b,c,d,e]Values interchangeable within column.

experiments showed that the bioactivity maximized in uv-active fractions, the ^{1}H-nmr spectra of which showed that each contained the same compound.

This was identified as the known compound 2-(3-methyl-2-butenyl)-1,4-naphthalenedione, deoxylapachol [**1**], by comparisons with the published ^{1}H-nmr, uv, and ir spectra (5–7). The ^{13}C-nmr spectrum of **1** has not been reported, but the data obtained were consistent with assignments published for lapachol [**2**] (8) and 2-methyl-1,4-naphthalenedione (9).

Deoxylapachol [**1**] has a P-388 IC_{50} of 0.6 μg/ml. The IC_{50} is the calculated concentration of a sample that would inhibit the growth of P-388 leukemia cells to 50% of the growth in the control (solvent only) well. Compound **1** also showed cytotoxicity against monkey kidney cells (BSC) at 10 μg/well, but did not inhibit the cytopathic effects of either *Herpes simplex* Type I or *Polio* Type I viruses (4). Disk assays on **1** (at 60 μg/disk) showed activity against the Gram-positive bacterium *Bacillus subtilis* and the fungi *Candida albicans* and *Trichophyton mentagrophytes* but not against the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*.

1,4-DIMETHOXY-2-(3-METHYL-2-BUTENYL)-NAPHTHALENE [**3**].—The ^{1}H- and ^{13}C-nmr spectra of the CH_2Cl_2-soluble fraction from the *L. quercifolia* extract showed the presence of a single major component. This compound, which fluoresced blue under uv light on Si gel tlc, was purified by Si gel cc. It was present at the remarkably high level of about 0.4% of the wet wt of the alga.

Hrms measurements supported a formula of $C_{17}H_{20}O_2$ for the molecular ion, in accord with the ^{1}H- and ^{13}C-nmr spectra (Table 1). These spectra (and a one-bond ^{1}H-^{13}C correlation spectrum) showed the presence of a 3-methyl-2-butenyl group, a one-proton singlet at 6.60 ppm, two methoxyl signals, and signals due to a 1,2-disubstituted benzene ring.

One structure consistent with these data was 1,4-dimethoxy-2-(3-methyl-2-butenyl)-naphthalene [**3**]. This proposed structure **3** was modelled by molecular mechanics methods, and conformational searches were made for the most stable rotamers about the C-1–O, C-2–C-1′, C-1′–C-2′, and C-4–O bonds. These predicted that the most stable conformations would have a C-8a–C-1–OMe dihedral angle of around +90° or −90°, whereas the C-4a–C-4–OMe dihedral angle would be about 170°. These were consistent with the observed nOe interactions, because only one methoxyl group showed nOe interactions with a proton on the disubstituted benzene ring (Figure 1). A similar difference between the orientations of ortho-disubstituted and ortho-

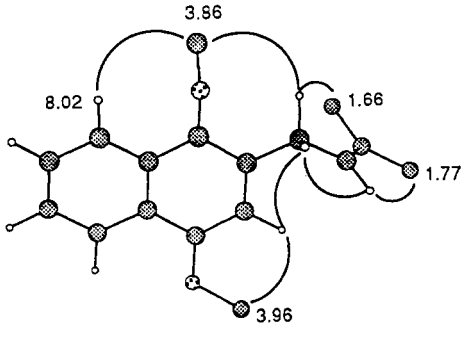
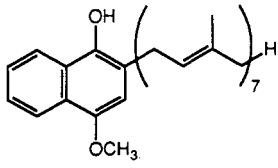


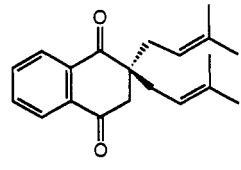
FIGURE 1. Compound **3** (methyl protons omitted) in one of the most stable conformations predicted by molecular mechanics calculations. NOe interactions are shown by solid lines.

monosubstituted aromatic methoxyl groups has been found in a crystal structure (10). The unusually high ^{13}C-nmr chemical shift of 1-OMe (61.91 ppm vs. the normal range of 55–57 ppm; see Table 1) also suggested that it was out of the aromatic ring plane, thus inhibiting methoxyl resonance (11).

This 1,4-dimethoxynaphthalene **3** has not previously been reported, although the 1,4-naphthoquinol **4** has been synthesized (no spectral data were given) (12). A fluorescent compound **5** from green photosynthetic bacteria had similar uv and ^{1}H-nmr spectra (13). Compound **3** was the major component of $CHCl_3$ extracts of freeze-dried samples of *L. quercifolia* from east and west coasts of the South Island and of a sample from the North Island, so it was not an artifact formed during extraction with MeOH. Compound **3** was not considered biologically active, with a P-388 IC_{50}>25 μg/ml.



5



6

2,3-DIHYDRO-2,2-BIS(3-METHYL-2-BUTENYL)-1,4-NAPHTHALENEDIONE [**6**].—A compound more polar than **3** was also obtained from the Si gel column and purified by Si gel tlc. The ^{1}H-nmr spectrum showed signals due to a 3-methyl-2-butenyl group with the methylene protons non-equivalent, a singlet at 2.91 ppm, and four mutually coupled aromatic protons (Table 1). Hrms measurements on the molecular ion (confirmed by fabms) were consistent with the formula $C_{20}H_{24}O_2$, with loss of C_5H_9 to give the base peak. These data could be accounted for by the presence of two symmetrically equivalent 3-methyl-2-butenyl groups. The integrals of the ^{1}H-nmr signals confirmed this, because the 3-methyl-2-butenyl "one-proton" signals and the singlet at 2.91 ppm were twice as large as the aromatic one-proton signals. The ir spectrum showed a carbonyl stretch at 1700 cm^{-1} (non-quinone), and the ^{13}C-nmr spectrum contained two carbonyl signals (Table 1). The only structure consistent with all these data was 2,3-dihydro-2,2-bis(3-methyl-2-butenyl)-1,4-naphthalenedione [**6**]. The methylene protons in each 3-methyl-2-butenyl side chain are diastereotopic because there is no molecular symmetry plane that can bisect these H-C-H groups, whereas the side chains are enantiotopic (14).

Compound **6** has not previously been reported as a natural product, but it was a byproduct of one synthesis of deoxylapachol [**1**] (7). The ^{1}H-nmr and ir spectra were compatible with those of the synthetic material, and the ^{13}C-nmr spectrum was assigned by comparison with that of **1** (Table 1). Compound **6** was inactive, with a P-388 IC_{50}>12.5 μg/ml.

2-(3-METHYL-2-BUTENYL)-2,3-EPOXY-1,4-NAPHTHALENEDIONE 4,4-DIMETHOXY KETAL [**7**].—Another compound was obtained from both the Si gel tlc and the C_{18} rp runs that yielded deoxylapachol [**1**]. Hrms (chemical ionization with isobutane) supported a molecular formula of $C_{17}H_{20}O_4$, and there was a facile loss of CH_4O. The ir spectrum showed a carbonyl stretch (1700 cm^{-1}), and the ^{1}H-nmr spectrum contained signals due to two methoxyl groups, a 3-methyl-2-butenyl group with diastereotopic methylene protons, a one-proton singlet at 3.86 ppm, and four mutally coupled aromatic protons (Table 1). ^{1}H-detected heteronuclear multiple-quantum coherence experiments, for both one-bond and two- or three-bond ^{1}H-^{13}C correlations (15), were used to establish the structure **7** of this compound. Key correlations were between both the

methoxyl proton signals and a quaternary carbon signal at 98.40 ppm, thus establishing the presence of a dimethoxy ketal. This ketal carbon was one of the substituents on the benzene ring, as its signal correlated with one of the aromatic proton signals. The other benzene ring substituent was the carbonyl group, as its carbon signal correlated with another aromatic proton signal. The remaining groups then had to constitute a trisubstituted epoxide, to give the structure 2-(3-methyl-2-butenyl)-2,3-epoxy-1,4-naphthalenedione 4,4-dimethoxy ketal [**7**].

This proposed structure was modelled by molecular mechanics methods, and conformational searches were made for the most stable rotamers about the two C-4–O bonds. These predicted that the most stable conformations would have the methoxyl group trans to the epoxide oxygen directed towards the aromatic ring, whereas the other methoxyl group would be directed away from the aromatic ring. These results were consistent with the observed nOe interactions (Figure 2).

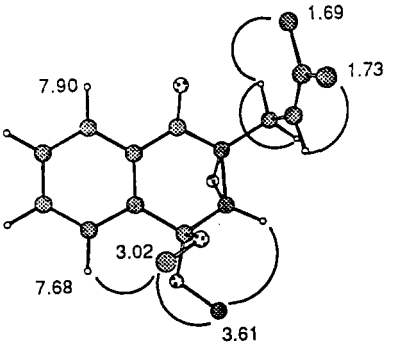
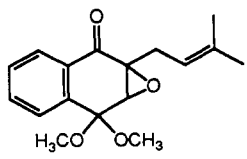


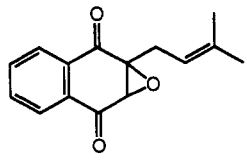
FIGURE 2. Compound **7** (methyl protons omitted) in one of the most stable conformations predicted by molecular mechanics calculations. NOe interactions are shown by solid lines.

Compound **7** has not previously been reported, but a search on the 2,3-epoxy-4,4-dimethoxycyclohexanone substructure retrieved a paper on a number of synthetic epoxynaphthoquinone ketals (16). These were obtained from 1,4-dimethoxynaphthalene derivatives of photooxygenation in MeOH solutions. Thus **7** could be an artifact derived from the major component 1,4-dimethoxy-2-(3-methyl-2-butenyl)-naphthalene [**3**], especially as **7** was a racemic mixture (no cd). Compound **7** was biologically inactive, with a P-388 $IC_{50}>12.5$ μg/ml.

2-(3-METHYL-2-BUTENYL)-2,3-EPOXY-1,4-NAPHTHALENEDIONE [**8**].—A $CDCl_3$ solution of ketal **7** was hydrolyzed by shaking with D_2O plus CF_3CO_2H, and the reaction was monitored by ^1H nmr. After several days, the methoxyl signals of **7** had almost completely disappeared, and a single major product was isolated. This was shown to be the known naphthoquinone epoxide **8** by its ms, uv, and ^1H-nmr spectra (17). Epoxide **8** has biological activities very similar to those of its parent naphthoquinone, deoxylapachol [**1**]. The IC_{50} of **8** against P-388 cells was 0.8 μg/ml (0.6 μg/ml for **1**), and it was also cytotoxic against monkey kidney cells at 10 μg/well, active against *B. subtilis*, *C. albicans*, and *Tr. mentagrophytes*, but not against *E. coli* or *P. aeruginosa*.



7



8

DISCUSSION

Neither deoxylapachol [**1**] nor any other naphthalene derivative has been reported from marine algae in the recent chemical literature, although prenylated quinone and phenol derivatives are common (18). However, it has been noted (without references) that: "... most algae produce the same types of quinones as are found in higher plants, including phylloquinone (vitamin K_1)" (19). Deoxylapachol [**1**] was originally isolated as a skin irritant from teak, the wood of the tree *Tectona grandis* (20), and it has been found in wood from other vascular plants (21).

Deoxylapachol [**1**] has not previously been reported to be cytotoxic or antifungal, but these biological activities have been reported for closely related naphthoquinones. Lapachol [**2**] has in vivo antitumor activity (22), and 1,4-naphthalenedione itself had a P-388 IC_{50} of 0.2 μg/ml and a range of antimicrobial activities similar to that of **1** in our assays. Antifungal activities have been reported for a range of naphthoquinones (23).

The most important molecular parameter affecting the in vivo antitumor activity of a series of 1,4-naphthoquinones was redox potential (24). Thus the lack of biological activity of compounds **3**, **6**, and **7** was not surprising, but the biological activity of epoxide **8** was unexpected. This could be due to intracellular reduction of **8** to **1**, analogous to the reduction of 2,3-epoxide of vitamin K_1 to vitamin K_1 which occurs during metabolism of this vitamin (25).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—All solvents were distilled before use and were removed by rotary evaporation at temperatures up to 45°. Reversed-phase flash chromatography followed the published method (26), and Davisil, 35–70 μm, 150 Å was used for Si gel flash chromatography. Tlc was done on Merck DC-Plastikfolien Kieselgel 60 F_{254}, visualized with a uv lamp. Mass, uv, and ir (film) spectra were recorded on Kratos MS80, Varian DMS 100, and Pye Unicam SP3-300 spectrometers, respectively. Nmr spectra, of $CDCl_3$ solutions at 23°, were recorded at 300 MHz for ^1H and 75 MHz for ^{13}C on a Varian XL300 spectrometer. Chemical shifts are given in ppm on the δ scale referenced to the solvent peaks: $CHCl_3$ at 7.25 and $CDCl_3$ at 77.00. Difference nOe experiments were done on non-degassed solutions using a low power cycling technique (27). Grid conformational searches and molecular mechanics calculations with standard MM2 parameters were performed using the MODEL/BAKMDL program 2.94, 1989, of Professor C. Still, extensively modified by Professor K. Steliou.

COLLECTION AND EXTRACTION.—*L. quercifolia* was collected from the Kaikoura Peninsula on the South Island of New Zealand in February 1989 (University of Canterbury voucher specimen 89K01-01). Frozen material (freed of epiphytes) (100 g) was extracted with MeOH (250 ml) followed by MeOH-CH_2Cl_2 (3:1) (2 × 200 ml) by blending and filtering. Solvent removal from a 1/20th aliquot gave a dark gum (0.45 g, IC_{50} 94 μg/ml). Most of the solvent was removed from the bulk extract, which was then partitioned between H_2O and CH_2Cl_2. Solvent removal from a 1/20th aliquot of the H_2O partition gave a pink and white solid (0.38 g, IC_{50} 400 μg/ml). Solvent removal from a 1/20th aliquot of the CH_2Cl_2 partition gave a brown gum (67 mg, IC_{50} 7 μg/ml). Frozen samples (10 g) of *L. quercifolia* from Kaikoura (89K01-01), from the Poor Knights Islands (174° 44.2′ E, 35° 28.3′ S) off the northeast coast of New Zealand (University of Canterbury voucher specimen 87PK03-07, collected in February 1987), and from Nancy Sound, Fiordland (University of Canterbury voucher specimen 89FL04-08, collected in October 1989) were freeze-dried (4 g dry wt for each) and extracted with $CHCl_3$ (50 ml, 2 × 25 ml) and the solvent removed to give yellow gums (29 mg, IC_{50} 36 μg/ml; 21 mg, IC_{50} 9 μg/ml; and 35 mg, IC_{50} 27 μg/ml,

respectively). Compound **3** was identified as the major component of each of these extracts by ^{1}H- and ^{13}C-nmr spectroscopy.

2-(3-METHYL-2-BUTENYL)-1,4-NAPHTHALENEDIONE, DEOXYLAPACHOL [**1**].—C_{18} rp flash chromatography on half of the CH_2Cl_2 partition from the bulk extract (0.7 g, precoated on 2 g C_{18}, loaded on a 20 g column), developed in steps from MeOH-H_2O (3:2) to CH_2Cl_2, led to recovery of most of the bioactivity in fractions eluted with MeOH-H_2O (9:1) (0.38 g, IC_{50} 7 μg/ml). A fraction eluted with MeOH was largely fucosterol (61 mg), the usual major sterol of the Phaeophyta (28,29). Phenyl rp flash chromatography on the combined actives was not useful in concentrating the activity. A subsample (28 mg) of the active fraction from this phenyl column was separated by tlc [three 10 cm × 10 cm sheets, C_6H_6-petroleum ether (2:1)]. A uv-active, pale yellow band at R_f 0.25 yielded **1** as a yellow oil (1 mg, IC_{50} 0.6 μg/ml). Rplc [Merck Lobar 31 cm × 2.5 cm column, stationary phase Lichroprep RP-18 40-63 μm, mobile phase MeOH-H_2O (4:1) at 8 ml/min] on another subsample (50 mg) gave a peak (254 nm detection) at 7 column volumes which yielded **1** as a yellow gum (4 mg, IC_{50} 0.6 μg/ml): eims 227 (15%), $[M]^+$ 226.0994 (66) ($C_{15}H_{14}O_2$ requires 226.0994), 212 (15), 211.0775 (100) ($C_{14}H_{11}O_2$ requires 211.0759), 183 (10), 165 (16), 128 (13), 115 (17), 105 (28), 104 (15), 89 (38), 87 (19), 77 (29), 76 (39), 75 (12), 73 (14); uv (MeOH) 325 nm (log ε 3.38), 257 (4.15), 250 (4.19), 246 (4.18); ir 2980, 2940, 2870, 1705, 1670, 1630, 1600, 1340, 1310, 1275, 790 cm^{-1}; ^{1}H and ^{13}C nmr see Table 1.

1,4-DIMETHOXY-2-(3-METHYL-2-BUTENYL)-NAPHTHALENE [**3**].—Si gel flash chromatography was carried out on half of the CH_2Cl_2 partition (0.7 g, precoated on 2 g silica, loaded on a 22 g column) developed in steps from petroleum ether to C_6H_6 to EtOAc. One group of fractions [petroleum ether-C_6H_6 (4:1), 148 mg, IC_{50} 33 μg/ml] was mainly one component by tlc [petroleum ether-C_6H_6 (1:1), fluorescent spot at R_f 0.6]. A subsample (16 mg) was further purified by preparative tlc [petroleum ether-C_6H_6 (1:1)] to give **3** as a pale yellow oil (12 mg): eims $[M]^+$ 256.1465 (100%) ($C_{17}H_{20}O_2$ requires 256.1463), 241 (30), 199 (45), 165 (30), 128 (25), 115 (25); uv (MeOH) 300 nm (log ε 4.07), 260 (4.13), 254 (4.23), 238 (4.89), 216 (4.77); uv (petroleum ether) 330 nm (log ε 3.61), 300 (3.84), 245 (4.72); ir 3090, 2950, 2850, 1600, 1470, 1370, 1270, 1230, 1090, 1010, 770 cm^{-1}; ^{1}H and ^{13}C nmr see Table 1.

2,3-DIHYDRO-2,2-BIS(3-METHYL-2-BUTENYL)-1,4-NAPHTHALENEDIONE [**6**].—A later group of Si gel column fractions [petroleum ether-C_6H_6 (1:4) to C_6H_6, 33 mg, IC_{50} 2 μg/ml] was mainly one component by tlc [petroleum ether-C_6H_6 (1:1), spot at R_f 0.2]. A subsample (7 mg) was further purified by preparative tlc [developed twice with petroleum ether-C_6H_6 (1:2)] to give **6** as a colorless oil (1.8 mg, IC_{50}>12.5 μg/ml): eims $[M]^+$ 296.1787 (17%) ($C_{20}H_{24}O_2$ requires 296.1776), 228 (24), 227.1078 (100) ($C_{15}H_{15}O_2$ requires 227.1071), 185 (25), 173 (15), 157 (15), 77 (20), 69.0715 (40) (C_5H_9 requires 69.0704); fabms 297 (30%), 229 (100), 228 (45), 227 (65), 173 (70); cims (isobutane) 298 (45%), 297 (100), 296 (30), 230 (30), 229 (100), 228 (30), 227 (95); uv (MeOH) 290 nm (shoulder), 250 (log ε 3.88), 225 (4.29); uv (petroleum ether) 305 nm (log ε 3.17), 295 (3.20), 248 (4.01), 224 (4.43); ir 3000, 2950, 2880, 1700, 1600, 1290, 1270, 760 cm^{-1}; ^{1}H and ^{13}C nmr see Table 1.

2-(3-METHYL-2-BUTERNYL)-2,3-EPOXY-1,4-NAPHTHALENEDIONE 4,4-DIMETHOXY KETAL [**7**].—The tlc plates that yielded deoxylapachol **1** also had uv-active bands at R_f 0.1, which yielded **7**, a colorless oil (1 mg, IC_{50} 15 μg/ml). The rplc run which yielded **1** had a peak at 4.5 column volumes which yielded **7**, a pale yellow oil (9 mg, IC_{50} 7 μg/ml); cims (isobutane) 290 (20%), $[MH]^+$ 289.1447 (96) ($C_{17}H_{21}O_4$ requires 289.1440), 258 (17), 257.1173 (100) ($C_{16}H_{17}O_3$ requires 257.1178), 256 (50), 225 (16), 223 (18), 195 (23); uv (MeOH) 290 nm (shoulder), 251 (log ε 4.17), 205 (4.55); ir 2940, 1700, 1610, 1465, 1300, 1195, 1145, 1080, 1055, 965, 775, 740 cm^{-1}; ^{1}H and ^{13}C nmr see Table 1.

2-(3-METHYL-2-BUTENYL)-2,3-EPOXY-1,4-NAPHTHALENEDIONE [**8**].—Compound **7** (2 mg) was dissolved in $CDCl_3$ (0.5 ml) and shaken in an nmr tube with D_2O (0.1 ml) and CF_3CO_2H (3 drops). After 12 days at 23°, tlc [petroleum ether-C_6H_6 (1:2)] showed a uv-active band at R_f 0.25 which yielded **8**, a colorless oil (0.9 mg, IC_{50} 0.8 μg/ml): eims $[M]^+$ 242.0945 (25%) ($C_{15}H_{14}O_3$ requires 242.0943), 227.0705 (78) ($C_{14}H_{11}O_3$ requires 227.0708), 214 (36), 213.0928 (100) ($C_{14}H_{13}O_2$ requires 213.0915), 199 (20), 196 (47), 195 (45), 181 (22), 173 (17), 171 (67), 167 (19), 159 (39), 158 (27), 152 (18), 115 (19), 105 (40), 104 (20); uv (MeOH) 300 nm (shoulder), 261 (log ε 3.67), 226 (4.25); ^{1}H nmr see Table 1.

ACKNOWLEDGMENTS

We thank PharmaMar SA for financial support for this work. We also thank C.N. Battershill and M.J. Page for collections and taxonomy, E.G. Becker for extractions, G. Barns and F.M. Dale for biological assays, B.M. Clark for MS, C. Still and K. Steliou for the MODEL/BAKMDL program, and H. Wynberg for additional information on epoxides.

LITERATURE CITED

1. M.H.G. Munro, J.W. Blunt, G. Barns, C.N. Battershill, R.J. Lake, and N.B. Perry, *Pure Appl. Chem.*, **61**, 529 (1989).
2. V.W. Lindauer, V.J. Chapman, and M. Aiken, "The Marine Algae of New Zealand II. Phaeophyceae," Verlag von J. Cramer, Weinheim, 1961, p. 302.
3. B. Czeczuga and F.J. Taylor, *Biochem. Syst. Ecol.*, **15**, 5 (1987).
4. M.H.G. Munro, R.T. Luibrand, and J.W. Blunt, in: "Bioorganic Marine Chemistry." Ed. by P.J. Scheuer, Springer-Verlag, Berlin, 1987, Vol. 1, p. 93.
5. N. Jacobsen and K. Torssell, *Acta Chem. Scand.*, **27**, 3211 (1973).
6. H. Inouye, T. Hayashi, and T. Shingu, *Chem. Pharm. Bull.*, **23**, 392 (1975).
7. N.K. Kapoor, R.B. Gupta, and R.N. Khanna, *Indian J. Chem.*, **21B**, 189 (1982).
8. B.A. Dawson, M. Girard, D. Kindack, J. Fillion, and D.V.C. Awang, *Magn. Reson. Chem.*, **27**, 1176 (1989).
9. V.P. Papageorgiou, *Planta Med.*, **40**, 305 (1980).
10. H.-H. Bartsch, H.W. Schmalle, O.H. Jarchow, B.M. Hausen, and K.-H. Schulz, *Acta Crystallogr., Sect. C*, **42**, 322 (1986).
11. E. Kiehlmann and A.S. Tracey, *Magn. Reson. Chem.*, **26**, 204 (1988).
12. E. Leistner, *Phytochemistry*, **12**, 337 (1973).
13. R. Powls, *FEBS Lett.*, **6**, 40 (1970).
14. W.B. Jennings, *Chem. Rev.*, **75**, 307 (1975).
15. M.F. Summers, L.G. Marzilli, and A. Bax, *J. Am. Chem. Soc.*, **108**, 4285 (1986).
16. L.A. Paquette, F. Bellamy, M.C. Bohm, and R. Gleiter, *J. Org. Chem.*, **45**, 4913 (1980).
17. H. Pluim and H. Wynberg, *J. Org. Chem.*, **45**, 2498 (1980).
18. D.J. Faulkner, *Nat. Prod. Rep.*, **5**, 613 (1988), and previous reviews in this series.
19. R.A. Lewin, in: "Algal Physiology and Biochemistry." Ed. by W.D.P. Stewart, Blackwell Scientific, Oxford, 1974, p. 17.
20. W. Sandermann and M.H. Simatupang, *Chem. Ber.*, **96**, 2182 (1963).
21. R.H. Thomson, "Naturally Occurring Quinones III: Recent Advances," Chapman and Hall, New York, 1987, p. 130.
22. K.V. Rao, *Cancer Chemother. Rep., Part 2*, **4**, 11 (1974).
23. H. Gershon and L. Shanks, *Can. J. Microbiol.*, **21**, 1317 (1975).
24. E.M. Hodnett, C. Wongwiechintana, W.J. Dunn III, and P. Marrs, *J. Med. Chem.*, **26**, 570 (1983).
25. J.W. Suttie and P.C. Preusch, *Haemostasis*, **16**, 193 (1986).
26. J.W. Blunt, V.L. Calder, G.D. Fenwick, R.J. Lake, J.D. McCombs, M.H.G. Munro, and N.B. Perry, *J. Nat. Prod.*, **50**, 290 (1987).
27. N.B. Perry, J.W. Blunt, and M.H.G. Munro, *Magn. Reson. Chem.*, **27**, 624 (1989).
28. A.G. McInnes, J.A. Walter, and J.L.C. Wright, *Org. Magn. Reson.*, **13**, 302 (1980).
29. V. Amico, G. Oriente, M Piatelli, C. Tringali, E. Fattorusso, S. Magno, L. Mayol, C. Santacroce, and D. Sica, *Biochem. Syst. Ecol.*, **4**, 143 (1976).

Received 5 November 1990