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PHENOLIC LIPIDS FROM RELATED MARINE ALGAE OF THE ORDER DICTYOTALES

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Key Word Index—*Zonaria farlowii*; *Z. diesingiana*; *Lobophora papenfussii*; Phaeophyta; Dictyotaceae; brown algae; phenolic lipids; phloroglucinol; δ-tocopherol; marine antibacterial agents.

Abstract—2-(1′-Oxo-dodeca-5′,8′,11′,14′,17′(all *Z*)-pentaenyl)-5-methoxy-1,3-dihydroxybenzene, 2-(1′-oxo-dodeca-5′,8′,11′,14′,17′(all *Z*)-pentaenyl)-1,3,5-trihydroxybenzene, 2-(17′-hydroxy-1′-oxo-dodeca-5′,8′,11′,14′(all *Z*)-tetraenyl)-1,3,5-trihydroxybenzene and 2-(1′oxo-hexadecyl)-1,3,5-trihydroxybenzene have been isolated from the related brown algae *Zonaria farlowii*, *Z. diesingiana* and *Lobophora papenfussii*. The structures of these new metabolites are based on extensive spectral analyses and comparisons with model compounds. The isolation of (+)-7,8-dimethyltocol, from *L. papenfussii*, is also reported.

INTRODUCTION

Many marine algae of the order Dictyotales are rich sources of novel terpenoids[1], several of which possess exciting pharmacological and other biological properties[2]. In our quest for new bioactive metabolites from algae of this order, we have examined the lipid components of several related algae; *Zonaria farlowii* (Setchell and Gardner) from the southern California coast, *Z. diesingiana** (J. Agardh) from Fukuoka Harbor, Japan, and *Lobophora papenfussii** (Taylor) Womersley from Palau, Micronesia. From these three algae we have isolated several similar acetate-derived metabolites as the major components of the lipid extractable material. These compounds show varying degrees of *in vitro* antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* (Table 2). It is interesting that these new phenolic lipids are homologous with **11**, a metabolite recently isolated from the taxonomically unrelated brown alga *Cystophora torulosa* (order: Fucales; family: Sargassaceae)[3]. Herein, we report the isolation and structure determination of these new metabolites as **1–4**, as well as the isolation of the previously described compound, (+)-7,8-dimethyltocol (**5**).

RESULTS AND DISCUSSION

Z. farlowii, an abundant shallow water alga of southern California and its offshore islands, was collected by hand from several areas within its geographical range. Open column Si gel chromatography of the chloroform extract yielded fractions containing **1**, which when purified by HPLC was isolated as a light-yellow oil. Electron impact mass spectrometry indicated a small parent ion at *m/z* 424, thus suggesting a molecular formula of $C_{27}H_{36}O_4$. The ^{1}H NMR spectrum of **1** indicated that the majority of protons in the molecule formed a contiguous homo-conjugated polyene system which terminated at one end with a vinyl ethyl group. Five of the ten degrees of unsaturation inherent in the molecular formula were thus accounted for by this non-conjugated polyene system. Two high field aromatic protons appeared as a singlet at δ 5.73. These data, taken in consideration with the unusual ^{13}C NMR bands at 165.9 (1C,*s*), 163.8 (2C,*s*), 104.9 (1C,*s*) and 94.4 (2C,*d*), indicated that **1** possessed a 2-alkylated-1,3,5-benzene-triol functionality, thereby accounting for an additional four degrees of unsaturation. The remaining degree of unsaturation was identified as a carbonyl (a ^{13}C NMR band at 206.5*s*) in conjugation with the aromatic ring ($\gamma_{C=O} = 1610\ cm^{-1}$)[4, 5]. Aromatic methoxyl resonances at 55.4(*q*) in the ^{13}C NMR and δ 3.70(3H,*s*) in the ^{1}H NMR spectra were assigned to the C-5 position to fulfill the requirement of symmetry observed in the ^{13}C NMR and ^{1}H NMR spectra. The methylene protons α to the carbonyl were observed as a sharp triplet at δ 3.02(2H,$J = 7$ Hz), and the remaining two highfield methylenes at C-3′ and C-4′ were located via spin-decoupling experiments. Acetylation produced the expected symmetrical diacetate **6**. The all *Z* orientation of the side chain olefins is proposed for **1** based upon the lack of significant absorption between 800 and 900 cm^{-1} in its IR spectrum [6].

From slightly more polar fractions of the *Z. farlowii* extract, as well as from the *Z. diesingiana* extract, the major metabolite **2** was isolated as a pale yellow oil. Examination of the ^{1}H NMR, ^{13}C NMR and IR spectra (Table 1) coupled with the MW of 410 (corresponding to a formula of $C_{26}H_{34}O_4$), by low resolution electron impact, chemical ionization (NH_3, CH_4) and field desorption mass spectrometry, indicated **2** to be the triol precursor of **1**. Acetylation of

*Specimens of these two algae, identified by Dr. James N. Norris, have been deposited in the National Herbarium of the Smithsonian Institution, Washington, D. C., U.S.A.

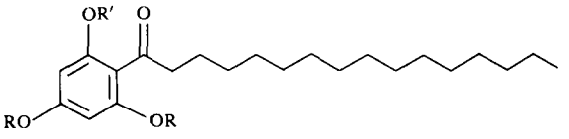
Table 1. ^1H (220 MHz) and ^{13}C (20 MHz) NMR data for the products **1–4**

	1		**2**		**3**		**4**	
Carbon no.	^{13}C $(CD_3)_2CO$	^1H CCl_4	^{13}C $CDCl_3$	^1H C_6H_6	^{13}C $CDCl_3$	^1H CD_3OD	^{13}C $CDCl_3$	^1H
C-1′	206.5*s*		205.8*s*		206.5*s*		207.5*s*	
C-5	165.9*s*		164.9*s*		164.0*s*		165.7*s*	
C-1, C-3	163.8*s*		164.6*s*		163.1*s*		165.7*s*	
Homoallylic	132.1*d*	5.43	132.1*d*	5.29	131.5*d*	5.23		
olefins*	129.7*d*	(10H, *m*)	130.1*d*	(10H, *m*)	129.6*d*	(8H, *m*)		
	128.7*d*		128.8*d*		128.5*d*			
	128.4*d*		128.7*d*		127.7*d*			
	128.2*d*		128.5*d*		125.2*d*			
	127.0*d*		128.3*d*					
			127.6*d*					
C-2	102.7*s*		105.0*s*		104.9*s*		105.4*s*	
	94.4*d*	5.92	95.6*d*	5.73	95.7*d*	5.89	95.7*d*	5.92
C-4, C-6		(2H, *s*)		(2H, *s*)		(2H, *s*)		(2H, *s*)
C-17					72.2*d*	3.75		
						(1H, *tt*, J = 6,6 Hz)		
		3.70						
OMe	55.4*q*	(3H, *s*)						
C-2′	43.6*t*	3.02	43.6*t*	3.08	43.4*t*	3.07	44.8	3.06
		(2H, *t*, J = 7 Hz)		(2H, *t*, J = 7 Hz)		(2H, *t*, J = 8 Hz)		(2H, *t*, J = 7.3 Hz)
C-16′					38.6*t*	2.27	14.5*q*	0.88
						(2H, *dd*, J = 6,6 Hz)		(3H, *t*, J = 6.5 Hz)
Unassigned	30.0*t*	2.88	29.8*t*	2.78	35.0*t*	2.83	33.0*t*	2.05
methylene	27.1*t*	(8H, *m*)	27.4	(8H, *m*)	27.0*t*	(6H, *m*)	30.7*t*	(2H, *m*)
groups*	25.8*t*		26.0		25.9*t*	2.14	26.2*t*	1.66
	24.8*t*	(2H, *dt*, J = 7,7 Hz)	25.1	(2H, *dt*, J = 7,7 Hz)	25.8*t*	(2H, *m*)	23.7*t*	(2H, *m*)
	20.7*t*		20.9		24.7*t*			
		2.00		2.04	18.9*t*	1.76		1.28
		(2H, *m*)		(2H, *m*)		(2H, *m*)		(22H, *m*)
		1.80		1.80		1.53		
		(2H, *dt*, J = 7,7 Hz)		(2H, *dt*, J = 7,7 Hz)		(4H, *m*)		
	14.2*q*	0.93	14.4*q*	0.96	14.0*q*	0.96		
C-20′		(3H, *t*, J = 7 Hz)		(3H, *t*, J = 7 Hz)		(3H, *t*, J = 7 Hz)		

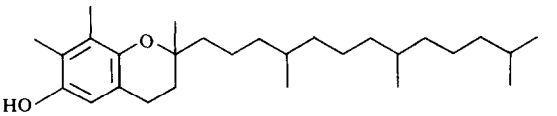
*Several bands were overlapping in this region of the spectra.

1 R′ = Me, R = H
2 R′ = R = H
6 R′ = Me, R = Ac
7 R′ = R = Ac

3



4 R′ = R = H
8 R′ = H, R = Me
9 R′ = Ac, R = Me
10 R′ = R = Ac



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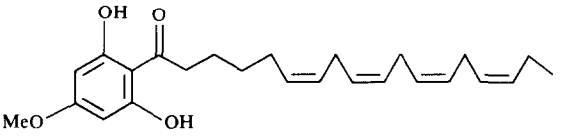

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2 led to the exclusive production of the triacetate **7** which showed strong aromatic acetate carbonyl absorptions in the IR spectrum ($\gamma_{C=O} = 1745\ cm^{-1}$).

From the *Z. diesingiana* extract, a more polar compound, **3**, was isolated as a minor component. Compound **3** was analysed for $C_{26}H_{36}O_5$ by low resolution mass spectrometry, and recognized as a side chain secondary alcohol by its characteristic ^1H and ^{13}C NMR features (Table 1). By ^1H spin-decoupling, the alcohol could be confidently placed at C-17′. Also, three sequentially coupled methylene groups were located at C-2′ through C-4′, which securely placed the homoallylic tetraene between C-5′ and C-15′. Thus, the alcohol **3** was assigned as the C-17′–C-18′ hydration product of the pentaene **2**.

From the related alga *Lobophora papenfussii*, two UV-absorbing compounds, **4** and **5** were isolated using conventional Si gel chromatography. Compound **4** was analysed for $C_{22}H_{36}O_4$ by low resolution field desorption mass spectrometry and was assigned as the saturated homologue of metabolite **2** by consideration of ^1H and ^{13}C NMR features. All proton and carbon bands due to the *ortho*-acyl phloroglucinol functionality were present and the remainder of the signals in the ^1H NMR spectrum were high field methylenes, most of which came at the same chemical shift of δ 1.28(22H,*s*). From the molecular formula and ^1H NMR integration data, **4** was shown to possess the saturated C_{16} side chain.

Interestingly, when **4** was treated with CH_2N_2, the dimethoxy derivative **8** was the exclusive product. In the ^1H NMR spectrum of this product the aromatic protons were rendered non-equivalent [δ 6.06(1H,*d*, $J = 2$ Hz) and δ 5.91(1H,*d*, $J = 2$ Hz)]. Presumably, the lack of methylation of one *ortho* hydroxyl group reflects the stabilization gained through hydrogen bonding with the side chain carbonyl group. Acetylation of **8** however, produced the acetate **9** which was accompanied by the expected shift of the adjacent aromatic ring proton [δ 6.39(1H,*d*, $J = 2$ Hz); δ 6.26(1H,*d*, $J = 2$ Hz)]. For comparison, the triol **4** was acetylated to yield the triacetate **10**, and in this case both protons were equivalent and observed as a 2H singlet at δ 6.93.

From less polar fractions of the *L. papenfussii* extract, the known tocol derivative **5**, (+)-7,8-dimethyltocol was isolated. Compound **5** showed $[\alpha]_D + 2.75°$ and was identical to an authentic sample and to published spectral features [7].

The isolation of these specific metabolites from several algae of the family Dictyotaceae, and from the taxonomically unrelated alga *Cystophora torulosa* [3], suggests that these compounds are of a more widespread distribution. A reasonable biosynthesis for these metabolites has already been proposed [3].

EXPERIMENTAL

General. ^1H NMR spectra were recorded at 220 and 60 MHz and ^{13}C NMR spectra at 20 MHz (all chemical shifts

Table 2. Zones of inhibition (in mm) of microbial growth using 6 mm diameter application discs

Compound	Microorganisms						
	Staphylococcus aureus	*Bacillus subtilus*	*Escherichia coli*	*Enterobacter aerogenes*	*Pseudomonas aeruginosa*	*Candida albicans*	*Vibrio anguillarum*
1	20	7	—	—	—	—	—
2	8	14	—	—	—	—	—
3	8	8	—	—	—	—	—
4	—	—	—	—	—	—	—

are reported relative to TMS). FD/MS were obtained in the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois, supported in part by a grant from the National Institute of General Medical Sciences (GM 27029). CI/MS were obtained at the Marine Biomedical Institute, University of Texas, Medical Branch, Galveston.

Collection, extraction and chromatography. 3.5 kg fresh *Z. farlowii*, collected intertidally in La Jolla, California in Nov. 1976, was extracted with $CHCl_3$–MeOH and fractionated (40 g) over Si gel to yield **1** and then **2**. Insidious green chlorophyll degradation products were removed from these fractions by absorption on activated charcoal to provide pure **1** (880 mg, 2.2%) and **2** (4.4 g 11%). *Z. diesingiana* was collected in shallow water (1–2 m) from outside Fukuoka Harbor, Japan in Sept. 1979, and stored in IPA for 3 weeks prior to $CHCl_3$–MeOH (2:1) extraction (8 g yield). Conventional Si gel CC gave fractions containing **2** (420 mg, 5.3%) and **3**. HPLC purification of fractions containing **3** yielded 39.9 mg (0.5%). *L. papenfussii* was collected in shallow water (6–10 m) from the NW coast of Babelthuap Island, Palau, Micronesia in Sept. 1979, and preserved frozen until extraction with $CHCl_3$–MeOH (2:1). Si gel CC followed by HPLC purification, yielded pure **5** (14.5 mg, 0.003%) and then **4** (130 mg, 9.026%).

Acetylations. Identical procedures were used in the esterification of **1**, **2**, **4** and **8**. *Ca* 10 mg natural product was stirred overnight at room temp. in excess equal molar quantities of pyridine and Ac_2O. The reactions were quenched with ice and then H_2O and the products extracted into Et_2O. The Et_2O phase was washed with 5% HCl (3 × 30 ml), satd $NaHCO_3$ (3 × 30 ml) and then dried ($MgSO_4$). Filtration and removal of Et_2O *in vacuo* gave near quantitative yields of **6**, **7**, **9** and **10** which were pure by 220 MHz ^{1}H NMR analysis.

2-(1′-*Oxo-dodeca*-5′,8′,11′,14′,17′ (*all* Z)-*penta-enyl*)-5-*methoxy*-1,3-*dihydroxybenzene* (**1**). UV λ_{max}^{MeOH} = 286, ϵ = 16000; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3100, 2850, 1625, 1440, 1250, 1225, 1180, 1100; LRMS (20 eV, 300°) obs. *m/z* M$^+$ 424(0.5), 406(0.9), 360(0.6), 195(9.6), 182(50), 167(87), 79(83), 43(100); Acetate **6**: ^{1}H NMR (220 MHz, CCl_4) δ 6.50(2H, *s*), 5.32(10H, *m*), 3.80(3H, *s*), 2.80(8H, *m*), 2.61(2H, *t*, *J* = 7 Hz), 2.18(6H, *s*), 2.05(4H, *m*), 1.67(2H, *m*), 0.97(3H, *t*, *J* = 7 Hz); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3000, 1800, 1640, 1360, 1180, 1130; LRMS (15 eV) obs. *m/z* M$^+$ 508(2), 465(4), 422(4), 224(41), 182(100), 167(60), 129(28).

2 - (1′ - *Oxo - dodeca* - 5′, 8′, 11′, 14′, 17′ (*all* Z) - *pentaenyl*) - 1, 3, 5 - *trihydroxybenzene* (**2**). UV λ_{max}^{MeOH} = 285, ϵ = 15100; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3100, 2850, 1625, 1600, 1460, 1380, 1240, 1180, 1080; LRMS (70 eV, 300°) obs. *m/z* M$^+$ 410(1), 274(2), 181(13), 167(59), 153(100); LRCIMS (CH_4, 60°) *m/z* 451 [M + 41]$^+$, 439 [M + 29]$^+$, 411 [M + H]$^+$, 393 [M − OH]$^+$, 285, 243, 153; (NH_3, 60°) 411 [M + H]$^+$, 393 [M − OH]$^+$, 153, 127; LRFDMS (15 ma, 3.0 kV) obs. *m/z* 410; HRMS obs. *m/z* M$^+$ 410.2400 ($C_{26}H_{34}O_4$, −4.2 mamu dev. from calc.); Acetate **7**: ^{1}H NMR (220 MHz, CCl_4) δ 6.83 (2H, *s*), 5.24 (10H, *m*), 2.74(8H, *m*), 2.61(2H, *t*, *J* = 7 Hz), 2.18(3H, *s*), 2.12(6H, *s*), 2.00(4H, *m*), 1.65(2H, *m*). 0.96(3H, *t*, *J* = 7 Hz); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2850, 1800, 1625, 1550, 1380, 1200, 1130, 1060.

2 - (17′ - *Hydroxy* - 1′ - *oxo* - *dodeca* - 5′, 8′, 11′, 14 (*all* Z)-*tetraenyl*) - 1, 3, 5 - *trihydroxybenzene* (**3**). $[\alpha]_D$ − 1.36° ($CHCl_3$; *c* 1.25; UV λ_{max}^{MeOH} = 288, ϵ = 21100; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 2950, 1610, 1450, 1190, 1075, 830; LRMS (70 eV, 350°) obs. *m/z* M$^+$ 428 (0.05), 410(0.06), 399(0.09), 382(0.09), 367(0.12), 254(1.1), 167(6.4), 149(8.2), 127(15), 95(26), 83(36), 69(47), 55(67), 43(100).

2 - (1′ - *Oxo* - *hexadecyl*) - 1, 3, 5 - *trihydroxybenzene* (**4**). UV λ_{max}^{MeOH} = 286, ϵ = 23300; IR $\nu_{max}^{CHCl_3}$: 3300, 2950, 1610, 1450, 1180, 970, 825; LRFDMS (15 ma, 2.8 kV) obs. *m/z* obs. M$^+$ 364; Triacetate **10**: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 1790, 1690, 1610, 1425, 1370, 1175, 1115, 1050, 1025, 895; ^{1}H NMR (220 MHz, $CDCl_3$) δ 6.93(2H, *s*), 2.70(2H, *t*, *J* = 7 Hz), 2.28(3H, *s*), 2.25(6H, *s*), 1.28(22H, *s*), 0.86(3H, *t*, *J* = 7 Hz).

Methylation of **4**. Excess CH_2N_2 in Et_2O was cautiously added to **4** (11.2 mg, 0.031 mmol), in Et_2O, and the reaction was stirred for 10 hr at 25°. Excess CH_2N_2 was allowed to escape and the product, **8**, was recovered in quantitative yield (12 mg). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 2950, 1730, 1600, 1450, 1150, 1100, 905, 820; ^{1}H NMR (220 MHz, $CDCl_3$) δ 6.06 (1H, *d*, *J* = 2 Hz), 5.91(1H, *d*, *J* = 2 Hz), 3.85(3H, *s*), 3.80(3H, *s*), 2.98(2H, *t*, *J* = 7 Hz), 1.24(22H, *s*), 0.86(3H, *t*, *J* = 7 Hz). Acetate **9**: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3000, 1750, 1610, 1460, 1360, 1180, 1145, 1090; ^{1}H NMR (220 MHz, $CDCl_3$) δ 6.39(1H, *d*, *J* = 2 Hz), 6.27(1H, *d*, *J* = 2 Hz), 3.81(3H, *s*), 3.80(3H, *s*), 2.82(2H, *t*, *J* = 7 Hz), 2.23(3H, *s*), 1.27(22H, *s*), 0.91(3H, *t*, *J* = 7 Hz).

Antimicrobial bioassays. Antibacterial and antiyeast bioassays were performed using the standardized agar plate-assay disc method. The results of these tests with compounds **1–4** appear in Table 2. One mg of each compound was applied to each assay disc and the disc placed on a freshly inoculated agar surface. After 24–36 hr, zones of inhibition developed and these were noted by measuring the total diameter of inhibited growth (Table 2).

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