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DITERPENOID METABOLITES FROM PACIFIC MARINE ALGAE OF THE ORDER CAULERPALES (CHLOROPHYTA)

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Key Word Index—*Chlorodesmis fastigata*; *Caulerpa brownii*; *Tydemania expeditionis*; *Udotea argentea*; Chlorophyta; diterpenoids; *E,E*-1,4-diacetoxybutadiene group; NMR; biological activity.

Abstract—Chemical investigations of two new species of *Udotea*, reinvestigation of *Tydemania expeditionis* and *Chlorodesmis fastigiata* from Guam and Saipan, Marianas Islands, as well as studies of *Caulerpa brownii* from Southern Australia, have led to the isolation of four new related diterpenoids and several previously described metabolites. These metabolites possess antimicrobial activities and inhibit cell division in the fertilized sea urchin egg bioassay.

INTRODUCTION

As a continuation of our chemical studies of tropical green algae of the order Caulerpales, we had a recent opportunity to investigate several Pacific representatives of this algal group collected in the Marianas Islands and in Australia. We report here the isolation of four new diterpenoids from *Chlorodesmis fastigiata* (C. Agardh) Ducker, *Tydemania expeditionis* W. v. Bosse, *Udotea argentea* Zanard, and *Caulerpa brownii* (C. Agardh) Endl. Several previously described diterpenoids were also components of these algae. The species studied are members of the related families Caulerpaceae and Udoteaceae which have been shown to produce metabolites possessing the unique *E,E*-1,4-diacetoxybutadiene functional group [1–8]. The new metabolites reported here also possess this functionality, the reactivity of which we believe explains the biological activity of these compounds. It should also be pointed out that the close chemical similarities between these two families of green algae may suggest a closer taxonomic relationship than is currently recognized.

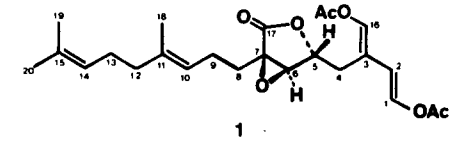
The biologically active compounds produced by many algal species within the Order Caulerpales appear to form the basis for a chemical defence adaptation within this group [3, 4, 8, 9, 18]. The algae are abundant in tropical and subtropical waters worldwide, and particularly in areas of intense predation due to herbivorous fishes and sea urchins. It has been recognized by numerous investigators that algae of the families Udoteaceae and Caulerpaceae are low preference items in the diets of most tropical herbivores [10–15].

RESULTS AND DISCUSSION

Two species of the pantropical genus *Udotea* were investigated from Guam and Saipan. Terpenoid metabolites from several species of *Udotea* from the Caribbean Sea and the Mediterranean have been reported [3, 16, 17], but Pacific species of this genus have not been investigated. *Udotea geppii* Yamada was collected in several reef habitats on the island of Saipan. The organic extract was found to contain the known sesquiterpenoid flexilin [1] as the sole terpenoid present. Flexilin was first isolated from *Caulerpa flexilis* [1], but subsequently also from the Caribbean alga *Udotea conglutinata* [8].

Collections of *Udotea argentea* were made in Guam and in Saipan lagoon. Both collections contained the known *Udotea* diterpenoid, udoteal, as a major metabolite [3]. In addition, *Udotea argentea* from Guam contained a new diterpenoid assigned as the epoxylactone, **1**. Compound **1** analysed by HRMS for $C_{24}H_{32}O_7$ (*m/z* 432.2147 $[M]^+$) and this formula was supported by ^{13}C NMR spectral features. Spectral evidence for the presence of the *E,E*-1,4-diacetoxybutadiene functionality included a UV λ_{max} at 245 nm (ε10400) and ^1H NMR signals at δ7.35 (1H, *d*, J = 12.6 Hz), 7.21 (1H, *s*), and 5.82 (1H, *d*, J = 12.6 Hz), which agree with published values from other compounds possessing this functional group [1–8]. Carbon-13 NMR bands at 172.7 (*s*), 69.3 (*d*), 57.3 (*d*), 57.0 (*s*), and ^1H NMR features, indicated the presence of an α,β-epoxy-γ-lactone (Tables 1 and 2). This assignment was also supported by infrared absorptions at 1770 and 1760 cm^{-1}, all of which were closely related to several useful model compounds [19]. The two enol acetates, the epoxylactone and two other trisubstituted double bonds (Tables 1 and 2) accounted for the nine degrees of unsaturation inherent in this metabolite. A strong mass spectral fragment which analysed for $C_{10}H_{15}$ (44% base peak) indicated the presence of a terminal ten carbon terpene portion of the molecule. This conclusion was substantiated by ^{13}C NMR spectral features which were analogous to those of geraniol (Table 2) [19]. Therefore the epoxylactone constellation was positioned at C-5–C-7 and this assignment was subsequently supported by ^1H NMR decoupling studies. The relative stereochemistry of the epoxide was assumed to be *cis* and the relative stereochemistry at C-5–C-6 was determined as *trans* due to the observed small (*ca* 1 Hz) coupling constant characteristic of the *trans* epoxide functionality [20]. The C-10–C-11 olefin geometry was assigned the *E* configuration due to the characteristic shielded C-18 methyl signal at 16 ppm (Table 2) [21].

The Pacific alga *Tydemania expeditionis* has been investigated previously [22], however only preserved



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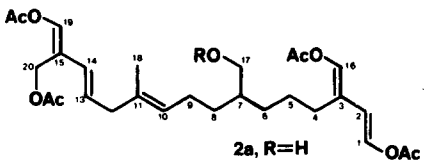
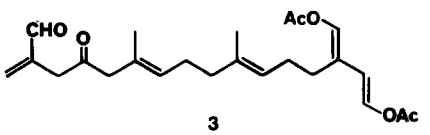


2a, R=H
2b, R=Ac



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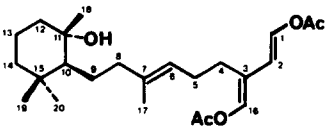

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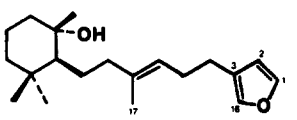

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samples were available for these prior studies. Since we had earlier shown that storage of these algae leads to decomposition in most cases, we chose to reinvestigate *T. expeditionis*. Investigation of freshly collected algae led to the isolation of a new diterpenoid (**2a**) possessing the bis-enol acetate functionality characteristic of other algae of the family Udoteaceae. Diterpenoid **2a** analysed for $C_{28}H_{40}O_9$ based upon HRMS and ^{13}C NMR spectral analyses. The UV spectrum (λ_{max} 243, ε11 000) and ^1H and ^{13}C NMR spectral values again indicated the presence of the bis-enol acetate functionality (Tables 1 and 2). Three other olefins and two acetate groups were also present in the compound, as shown by ^1H and ^{13}C NMR spectral features (Tables 1 and 2). In addition, a primary alcohol was indicated by appropriate ^1H and ^{13}C NMR bands [3.45 (*d*), 64.6 (*t*)]. The alcohol readily acetylated (Ac_2O–pyridine) to produce the pentaacetate **2b**, which resulted in the ^1H NMR spectrum in a consistent shift of the C-17 methylene protons to δ3.98.

The nine degrees of unsaturation inherent in the molecular formula of **2a** could be accounted for by consideration of all olefin and acetate unsaturation present in the molecule. Therefore, **2a** was concluded to be a linear diterpenoid. The subsequent positioning of all functional groups was based on ^1H NMR decoupling and NOE studies. The C-10 olefinic proton showed allylic coupling to both the C-18 methyl and C-12 bis-allylic methylene protons. The C-19 enol acetate proton showed allylic coupling to the C-20 methylene protons. The protons positioned at C-12 to C-14 were also readily interrelated by decoupling studies. The C-13–C-14 olefin geometry was determined as *E* based upon the observed 12.3 Hz coupling constant. The C-10–C-11 olefin geometry was established as *E* based on the shielded ^{13}C value of the C-18 methyl (Table 2) [21]. NMR studies

Table 1. ^1H NMR spectral data for diterpenoids **1**–**4** (360 MHz, $CDCl_3$)

H	1	2a	3	4
1	7.35 *d* (12.6)*	7.41 *d* (12.7)	7.34 *d* (12.5)	7.43 *d* (12.5)
2	5.82 *d* (12.6)	5.87 *d* (12.7)	5.86 *d* (12.5)	5.93 *d* (12.5)
4	2.20 *m*	2.59 *t* (7.6)	2.27 *m*	2.32 *m*
5	5.52 *dd* (9.3, 6.2)	1.36 *m*	2.11 *m*	2.19 *m*
6	3.41 *s*(*br*)	1.48 *m*	5.15 *t* (7)	5.17 *t* (7)
7	—	1.31 *m*	—	—
8	1.65 *m*	1.30 *m*	2.05 *m*†	1.73 *m*
9	2.05 *m*	1.99 *m*	2.00 *m*†	1.38 *m*†
10	5.09 *t* (7)	5.11 *t* (7)	5.22 *t* (7)	1.34 *m*†
12	2.02 *m*	2.72 *d* (6.7)	3.03 *s*	1.52 *m*†
13	1.98 *m*	5.68 *ddd* (12.3, 6.7, 6.7)	—	1.25 *m*†
14	5.02 *t* (7)	5.85 *d* (12.3)	3.26 *s*	1.20 *m*†
16	7.21 *s*	7.11 *s*	7.11 *s*	7.17 *s*
17	—	3.45 *d* (5.3)	1.61 *s*	1.62 *s*
18	1.57 *s*	1.57 *s*	1.61 *s*	1.16 *s*
19	1.62 *s*	7.26 *s*	9.44 *s*	0.82 *s*
20	1.66 *s*	4.79 *s*	6.28 *s*	0.94 *s*
			6.13 *s*	
OAc	2.18 *s*	2.17 *s*	2.12 *s*	2.17 *s*
	2.10 *s*	2.13 *s*	2.11 *s*	2.15 *s*
		2.11 *s*		
		1.99 *s*		

*J (Hz) given in parentheses.
†Values may be interchanged.

Table 2. ^{13}C NMR data for diterpenoids 1–5 [50 MHz, $CDCl_3$ (1 and 3), C_6D_6 (2, 4 and 5)]

C	1	2a	J_R (Hz)	3	J_R (Hz)	4	5
1	136.9 *d*	136.9 *d*	68	135.6 *d*	64	136.1 *d*	142.8 *d*
2	107.9 *d*	113.1 *d*	48	113.3 *d*	46	113.5 *d*	111.3 *d*
3	116.8 *s*	119.0 *s*‡	—	121.1 *s*	—	121.3 *s*	125.2 *s*
4	30.8 *t**	31.0 *t*†	—	26.6 *t*‡		27.1 *t*	28.8 *t*
5	69.3 *d*	25.2 *t*†		26.5 *t*‡		25.7 *t**	25.3 *t*
6	57.3 *d*	25.2 *t*†		123.6 *d*†	43	123.4 *d*	124.1 *d*
7	57.0 *s*	39.6 *d*	25	128.6 *s**	—	137.5 *s*	137.0 *s*
8	28.1 *t**	30.7 *t*†		39.2 *t*		43.8 *t*†	43.9 *t*†
9	26.6 *t**	25.4 *t*†		25.3 *t*‡		25.3 *t**	25.3 *t*
10	124.1 *d*	125.9 *d**	46	129.9 *d*†	42	57.0 *d*	56.7 *d*
11	136.4 *s*	133.2 *s*	—	135.6 *s**		73.8 *s*	73.7 *s*
12	39.6 *t*	43.1 *t*	28	40.3 *t*		41.9 *t*†	41.8 *t*†
13	22.9 *t**	126.6 *d**	46	205.1 *s*		20.9 *t*	20.8 *t*
14	122.7 *d*	129.1 *d*	56	54.0 *t*		43.4 *t*†	43.2 *t*†
15	131.4 *s*	121.1 *s*‡	—	143.4 *s*		35.7 *s*	35.6 *s*
16	133.4 *d*	135.6 *d*	70	134.3 *d*	63	135.0 *d*	139.2 *d*
17	172.7 *s*	64.6 *t*	33	16.3 *q*		16.5 *q*	16.2 *q*
18	16.0 *q*	15.8 *q*	25	15.9 *q*		23.6 *q*	23.5 *q*
19	17.7 *q*	34.4 *d*	69	193.2 *d*		21.7 *q*	21.6 *q*
20	25.6 *q*	56.6 *t*	39	136.8 *t*	50	33.1 *q*	33.0 *q*
OAc	167.5 *s*	169.8 *s*		167.8 *s*		167.5 *s*	
	166.6 *s*	167.1 *s*		167.4 *s*		167.0 *s*	
		166.4 *s*					
	2 × 20.6 *q*	166.3 *s*		2 × 20.7 q		2 × 20.2 *q*	
		20.0 *q*					
		2 × 19.6 *q*					
		19.5 *q*					

*†‡Values may be interchanged.

involving nuclear Overhauser enhancement difference spectral methods (NOEDS) showed that irradiation of the C-2 proton resulted in enhancement of the C-16 proton. This experiment demonstrated a transoid and coplanar geometry for the bis-enol acetate as already illustrated in several other metabolites of this class. Enhancement of the C-14 proton when the C-19 proton was irradiated indicated the *E,E* geometry of the conjugated C-13–C-14 and C-15–C-19 olefins.

The tropical Pacific alga *Chlorodesmis fastigiata* has also been previously investigated from the Australian Barrier Reef [7]. Our recent collection from the reef flat at Pago Bay, Guam, was found to contain two previously described *Chlorodesmis* metabolites, didehydrotrifarin and chlorodesmin [7], as 5% and 20% of the organic extract, respectively. The previously reported component, dihydrochlorodesmin, was not present in this collection. In addition, we have isolated a new, related diterpenoid, **3**, which composed approximately 10% of the organic extract. The aldehyde **3** analysed for $C_{24}H_{32}O_6$ by HRMS (m/z = 416.2193 $[M]^+$) and by interpretation of ^{13}C NMR spectral features. The overall spectral features of **3** again indicated the presence of the bis-enol acetate functionality (Tables 1 and 2). These latter NMR features, coupled with appropriate infrared absorptions, showed the presence of an aldehyde, a terminal methylene group, a ketone carbonyl, and two methyl-trisubstituted olefins (Tables 1 and 2). These functional groups accounted for all nine degrees of unsaturation inherent in the molecular formula; therefore **3** must be a linear diterpenoid.

Proton experiments involving NOEDS methods allowed the confident assignment of the final structure of **3**. Irradiation of the aldehyde proton produced enhancement of one terminal olefin proton (the *cis* C-20 proton). Irradiation of the C-14 methylene showed enhancement of the other C-20 terminal olefin proton. Subsequently, the C-10 olefinic proton was shown to be enhanced when the C-12 proton was irradiated. Again, the 1,4-diacetoxybutadiene functionality was determined to be transoid and coplanar by measurement of the enhancement of the C-2 proton upon irradiation of the proton at C-16. In a consistent fashion, both olefinic geometries (C-6–C-7 and C-10–C-11) were assigned as *E* based on the shielded ^{13}C values for the corresponding methyl groups [21].

Compound **3** is related to the known *Chlorodesmis* metabolite chlorodesmin through hydrolysis and loss of acetic acid. It was, therefore, conceivable that **3** was an artifact of the isolation procedure. However, we were unable to convert **2a** (structurally related to chlorodesmin) to a corresponding aldehyde under a variety of similar work-up conditions.

The genus *Caulerpa* occurs worldwide in tropical and subtropical waters and also in the temperate waters of Southern Australia. *Caulerpa brownii* was collected near Melbourne, Australia and our results were compared with prior investigations of this species. Blackman and Wells reported the diterpenoid caulerpol from a Tasmanian collection [23] and Capon *et al.* reported the lack of terpenoids in *C. brownii* collected in Western Australia

[6]. From our Melbourne collection of this alga, we have isolated the known diterpenoid metabolites trifarin and didehydrotrifarin [1, 7] as 5% of the organic extract combined. In addition, a new monocyclic diterpenoid alcohol, assigned as **4**, was isolated as the major metabolite (20% organic extract).

Compound **4** analysed for $C_{24}H_{38}O_5$ by combined HRMS and ^{13}C NMR spectral methods. The bis-enol acetate functionality was again shown to be present by the characteristic UV, ^1H NMR and ^{13}C NMR spectral features (Experimental and Tables 1 and 2). The infrared spectrum of **4** contained a hydroxyl band at 3400 cm^{-1} which was assigned to a tertiary hydroxyl group based upon ^{13}C NMR data (73.8 *s*). One other trisubstituted olefin was present as indicated by additional ^{13}C NMR features (Table 2). Based upon the six degrees of unsaturation inherent in the molecular formula, compound **4** must be a monocyclic diterpenoid. The subsequent assignment of the structure of this new diterpenoid was greatly aided by ^1H NMR decoupling studies using model compounds [24], and especially the comparison of NMR data to that from the sponge metabolite ambliol-A (**5**) [25]. Table 2 shows the excellent agreement of ^{13}C NMR values between these two compounds. Since the ^{13}C NMR values for carbons 10, 11, 18, 19 and 20 in **4** were almost identical to those from ambliol-A, the substituents at C-10 and C-11 were assigned the same relative stereochemistries (equatorial hydroxyl at C-11 and equatorial side-chain at C-10). As in numerous other metabolites, the olefin geometry at C-6–C-7 was assigned the *E* configuration based upon the shielded ^{13}C values for the C-17 methyl group (16.5 ppm) [21]. Absolute stereochemistry was not determined for compounds **4** or **5**.

All of the metabolites isolated from these Pacific algae have been extensively assayed for their biological activities [3, 8]. Compounds **1–4** show antibacterial activities toward the human pathogenic bacteria *Staphylococcus aureus* and *Bacillus subtilis*. Compounds **2a**, **3** and **4** were also tested against several marine bacteria and were found to be inhibitory toward *Vibrio harveyi* and *V. leiognathi*. Compound **4** is also active against *E. coli* and *V. anguillarum*. Compounds **1**, **2a** and **3** were tested for cytotoxicity in the fertilized sea urchin egg assay [26, 27]. All three compounds completely inhibit cell division of the fertilized eggs at a concentration of 8 μg/ml.

EXPERIMENTAL

General. ^1H NMR: 360 MHz Nicolet-Oxford Magnetics FT spectrometer; ^{13}C NMR: 50 MHz on a Nicolet FT-200 instrument. Algae were collected in various habitats around Guam and Saipan (April–June, 1983). The freshly collected plants were immediately ground and extracted (×2) with CH_2Cl_2. The extracts were reduced in volume and immediately chromatographed over Florisil using CH_2Cl_2–MeOH solvent mixtures. Final purification of metabolites was achieved by preparative silica HPLC with EtOAc–*iso*-octane solvent mixtures.

Epoxylactone **1**. Collections of *Udotea argentea* from Cetti Bay, Guam yielded **1** as a colourless oil (10% of organic extract) after silica gel HPLC (EtOAc–*iso*-octane, 1:4). Epoxylactone **1** showed $[\alpha]_D^{25} = +7.0°$ (c = 1.5, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2990, 1770, 1760, 1610, 1350, 1190, 1100; UV λ_{max}^{MeOH} 245 nm (ε10 400); HRMS *m/z*: 432.2147 $[M]^+$, calc. 432.2139. ^1H NMR (C_6D_6): δ7.58 (1H, *d*, J = 12.5 Hz), 7.24 (1H, *s*), 5.78 (1H, *d*, J = 12.5 Hz), 5.70 (1H, *m*), 5.21 (1H, *m*), 5.20 (1H, *m*), 2.85 (1H, *d*, J = 2 Hz), 2.27 (2H, *m*), 2.14 (2H, *m*), 2.07 (2H, *m*), 2.01 (2H, *m*), 1.84 (2H, *m*), 1.68 (3H, *s*), 1.62 (3H, *s*), 1.57 (6H, *s*), 1.41 (3H, *s*).

Tetraacetate **2a**. Collections of *Tydemania expeditionis* were made at several sites on the island of Guam but the algae was particularly abundant at Western Shoals in Apra Harbor. Compound **2a** was isolated as a colourless oil (5% of organic extract) after silica gel HPLC (EtOAc–*iso*-octane, 11:9). Tetraacetate **2** showed $[\alpha]_D^{25} = -0.8°$ (c = 1.5, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2990, 1750, 1740, 1350, 1100; UV λ_{max}^{MeOH} 243 nm (ε11 000); HRMS: 418.2363 $[M - C_4H_6O_3]^+$, calc. 418.2346.

Pentaacetate **2b**. Compound **2a** (10 mg) was treated with Ac_2O in C_5H_5N at room temp. for 30 min. The resulting pentaacetate showed the following ^1H NMR spectral features ($CDCl_3$): δ7.31 (1H, *d*, J = 12.5 Hz), 7.26 (1H, *s*), 7.12 (1H, *s*), 5.88 (1H, *d*, J = 15 Hz), 5.83 (1H, *d*, J = 12.5 Hz), 5.54 (1H, *ddd*, J = 15, 7, 7 Hz), 5.20 (1H, *t*, J = 6 Hz), 4.78 (2H, *s*), 3.98 (2H, *m*), 2.72 (2H, *d*, J = 7 Hz), 2.24 (4H, *m*), 2.18 (3H, *s*), 2.13 (3H, *s*), 2.11 (3H, *s*), 1.99 (3H, *s*), 1.98 (3H, *s*), 1.65 (2H, *m*), 1.58 (3H, *s*), 1.50 (2H, *m*), 1.30 (2H, *m*).

Diterpenoid aldehyde **3**. *Chlorodesmis fastigiata* was collected from wave-washed zones of the Pago Bay reef flat on Guam. Aldehyde **3** was isolated as a colourless oil (10% of organic extract) after silica gel HPLC (EtOAc–*iso*-octane, 1:4). Compound **3** showed $[\alpha]_D^{25} = 0°$ (c = 1.4, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2990, 1770, 1690, 1640, 1450, 1380, 1060; UV λ_{max}^{MeOH} 249 nm (ε15 800); HRMS *m/z*: 416.2193 $[M]^+$, calc. 416.2190: ^1H NMR (C_6D_6): δ9.17 (1H, *s*), 7.67 (1H, *d*, J = 12.5 Hz), 7.32 (1H, *s*), 5.88 (1H, *d*, J = 12.5 Hz), 5.75 (1H, *s*), 5.48 (1H, *s*), 5.20 (2H, *m*), 3.05 (2H, *s*), 2.89 (2H, *s*), 2.34 (2H, *m*), 2.24 (2H, *m*), 2.07 (2H, *m*), 2.02 (2H, *m*), 1.64 (3H, *s*), 1.62 (3H, *s*), 1.59 (3H, *s*), 1.58 (3H, *s*).

Diterpenoid alcohol **4**. Collections of *Caulerpa brownii* were made at Flinders Reef near Melbourne, Australia in May, 1984. Compound **4** was isolated as an oil (20% of organic extract) after silica gel HPLC (EtOAc–*iso*-octane, 7:13). Alcohol **4** showed $[\alpha]_D^{25} = -0.5°$ (c = 1.0, $CHCl_3$); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3400, 2950, 1720, 1350, 1200, 1080; UV λ_{max}^{MeOH} 249 nm (ε16 000); HRMS *m/z*: 304.2409 $[M - C_4H_6O_3]^+$; calc. 304.2403.

Bioassays. Antimicrobial assays were performed using the standard agar plate-assay disc method at disc concentrations of 100 μg. Zones of inhibited growth in excess of 4 mm were interpreted as positive. The effects of compounds on cell division were assessed using the fertilized eggs of the common Pacific sea urchin *Lytechinus pictus*. Details of this assay have been published elsewhere [27].

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