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DILOPHIC ACID, A DITERPENOID FROM THE TROPICAL BROWN SEAWEED *DILOPHUS GUINEENSIS*

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Key Word Index—*Dilophus guineensis*; Dictyotales; Phaeophyta; diterpenoid; xenicane.

Abstract—A new xenicane-type diterpenoid containing a carboxylic acid has been isolated from the tropical brown alga *Dilophus guineensis*. Its structure was established via spectroscopic arguments including the use of two-dimensional proton–proton and proton–carbon COSY experiments. This new diterpene shows slight Gram-positive antimicrobial activity and is weakly ichthyotoxic.

INTRODUCTION

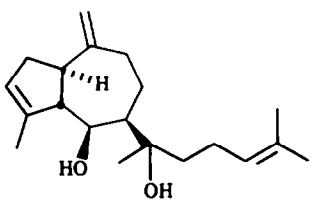
The brown algal order Dictyotales has been a rich source of diterpenes and to a lesser extent sesquiterpenes [1]. Many of these diterpenes represent 'prenylated' homologues of several known sesquiterpene classes. For example, germacrane, elemane, guiane, selinane, patchulane, bicyclogermacrane and bourbourane sesquiterpene skeletons have been shown to possess diterpene equivalents in this order [2–7, 16]. However, new classes of diterpenes have also been isolated from this group of algae which have no sesquiterpene equivalents [7–9], one of which is the xenicane system [2]. Although originally discovered from soft corals [10], these cyclononane derivatives have been isolated from species of both *Dictyota* [2, 12] and *Dilophus* [11] as well as from the herbivorous seahare *Aplysia depilans* [12].

As part of an exploration of Caribbean seaweed chemistry we have examined the lipid extracts of several algae from the order Dictyotales, including *Dilophus guineensis*. We have found this species of *Dilophus* to contain diterpenes of two skeletal classes, the dictyol and xenicane ring systems [13]. The dictyol type diterpene **1** was identified as dictyol E by comparison with published data [14] while the xenicane-type diterpene **2** was new and its structure established by spectroscopic methods, including several two dimensional NMR techniques. The occurrence of a relatively rare carboxylic acid functionality in this new diterpene has led to our proposing the trivial name dilophic acid for this new xenicane **2**.

RESULTS AND DISCUSSION

D. guineensis was collected from Vega Baja, Puerto Rico, where it grows abundantly in exposed habitats. A standard lipid extract (chloroform–methanol) of methanol-preserved seaweed was initially fractionated over silica gel in the vacuum mode and the new compound, dilophic acid **2**, was then further purified using HPLC (colourless oil, 7.5% of extract). Initial spectroscopic analysis revealed **2** to be strongly optically active and to possess only ketone end absorption in its UV spectrum. The IR spectrum showed a broad absorption between 2400 and 3600 cm^{-1} together with an intense 1715 cm^{-1} carbonyl peak. A molecular formula of $C_{20}H_{32}O_2$ was established by HREIMS ($[M]^+$ at *m/z* 304.2408, 0.0005 amu dev.) which was confirmed by elemental analysis. Of the five degrees of unsaturation inherent in this molecular formula three were accounted for by olefins and one by a carbonyl (see Table 1). The ^{13}C NMR chemical shift of this carbonyl (δ180.34), the occurrence of an exchangeable proton at δ11.8 in the ^1H NMR spectrum and the reactivity of **2** when treated with diazomethane to form a methyl ester **3** all confirmed the presence of a carboxylic acid in **2**, thus accounting for both oxygen atoms. Hence, **2** was deduced to be monocarbocyclic. Three partial structures (**A**–**C**) were deduced from an analysis of the high-field ^1H NMR and ^{13}C NMR data.

In partial structure **A**, two of the three olefins could be related to one another from coupling information ob-



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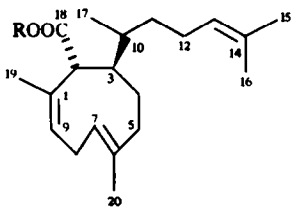
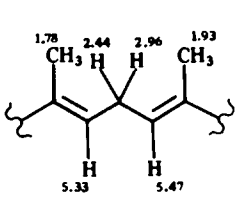


2 R = H
3 R = CH_3

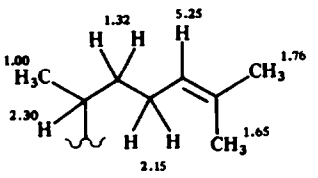
Table 1. NMR spectral data for dilophic acid (2)*

Assignment	^{1}H shift	Associated protons by COSY	^{13}C shift	Associated protons by long-range HETCOSY
1	—	—	140.78	1.85 (H-3); 2.21 H-19; 2.40 (H-8β); 3.71 (H-2)
2	3.71 (1H, *s*)	1.85 (H-3)	48.02	1.58 (H-4α); 1.85 (H-3); 5.46 (H-9)
3	1.85 (1H, *m*)	1.00 (H-17) 1.58 (H-4α) 2.10 (H-4β) 3.71 (H-2)	50.37	1.00 (H-17); 1.32 (H-11); 2.30 (H-10); 3.71 (H-2)
4α	1.58 (1H, *m*)	1.85 (H-3) 2.05 (H-5α) 2.10 (H-4β) 2.15 (H-5β)	28.34	1.85 (H-3); 3.71 (H-2)
4β	2.10 (1H, *m*)	1.58 (H-4α) 2.15 (H-5β)		
5α	2.05† (1H, *m*)	1.58 (H-4α) 2.15 (H-5β)	41.62	1.85 (H-3); 2.40 (H-8β); 5.46 (H-7)
5β	2.15† (1H, *m*)	1.58 (H-4α) 2.05 (H-5α) 2.10 (H-4β)		
6	—	—	135.04	1.58 (H-4α); 1.82 (H-20); 2.15 (H-5); 2.40 (H-8β); 2.95 (H-8α)
7	5.46 (2H, *br d*, J = 10.0 Hz)	1.82 (H-2O) 2.40 (H-8β) 2.95 (H-8α)	128.23	1.82 (H-20); 2.15 (H-5); 2.40 (H-8β)
8α	2.95 (1H, *ddq*, J = 12.7, 12.7 1.9 Hz)	2.21 (H-19) 2.40 (H-8β) 5.46 (H-7, H-9)	27.95	5.46 (H-7, H-9)
8β	2.40 (1H, *br dd*, J = 12.7, 7.3)	1.82 (H-20) 2.95 (H-8α) 5.46 (H-7, H-9)		
9	5.46 (1H, *br d*, J = 10 Hz)	2.21 (H-19) 2.40 (H-8β) 2.95 (H-8α)	126.59	2.21 (H-19); 2.95 (H-8α); 3.71 (H-2)
10	2.30 (1H, *tq*, J = 6.5, 6.5 Hz)	1.00 (H-17) 1.32 (H-11)	34.12	1.00 (H-17); 1.32 (H-11); 1.85 (H-3); 3.71 (H-2)
11	1.32 (2H, *dt*, J = 7.0, 7.0 Hz)	2.15 (H-12) 2.30 (H-10)	38.41	1.00 (H-17); 1.85 (H-3); 2.10 (H-4β)
12	2.15 (2H, *m*)	1.32 (H-11) 5.25 (H-13)	26.39	1.32 (H-11)
13	5.25 (1H, *br t*, J = 6.7 Hz)	1.65 (H-16) 1.76 (H-15) 2.15 (H-12)	125.25	1.32 (H-11); 1.65 (H-16); 1.76 (H-15); 2.15 (H-12)
14	—	—	131.26	1.65 (H-16); 1.76 (H-15); 2.15 (H-12)
15	1.76 (3H, *s*)	1.65 (H-16) 5.25 (H-13)	25.83	1.65 (H-16); 5.25 (H-13)
16	1.65 (3H, *s*)	1.76 (H-15) 5.25 (H-13)	17.73	1.76 (H-15); 5.25 (H-13)
17	1.00 (3H, *d*, J = 7.0 Hz)	1.85 (H-3) 2.30 (H-10)	15.56	1.32 (H-11); 1.85 (H-3); 2.30 (H-10)
18	—	—	181.12	1.85 (H-3); 2.21 (H-19); 3.71 (H-2)
19	2.21 (3H, *br s*)	5.46 (H-9) 3.71 (H-2)	22.43	5.46 (H-9)
20	1.82 (3H, *s*)	5.46 (H-7)	16.87	5.46 (H-7)

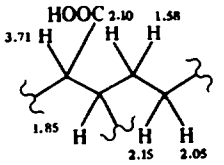
*Run on a Bruker AM 400 in benzene-d_6.
†Assignments may be reversed.



A



B



C

tained by a COSY experiment of **2** in $CDCl_3$. The olefin proton at δ5.33 was correlated to two midfield protons (δ2.44 and 2.96) that were themselves geminally coupled as well as being additionally correlated to another olefinic proton located at δ5.47. These two olefins accommodated two (δ1.78 and 1.93) of the four olefinic methyl groups which were identified from their respective long-range correlations to the olefinic protons.

Partial structure **B** was also deduced by analysis of the COSY data of **2** in benzene-d_6. A methine proton (δ2.30) appearing as a 1:5:10:10:5:1 sextet was coupled to both the doublet methyl group at high field (δ1.00) and a high-field methylene (δ1.32). These two protons appeared as a 1:3:3:1 quartet and were correlated with two allylic protons appearing as a multiplet at δ2.15. This latter signal was strongly correlated to the broadened triplet olefin proton at δ5.25 which was in turn allylically coupled to the remaining two olefinic methyl groups (δ1.65 and 1.76).

Partial structure **C** was again formulated from COSY data of **2** in benzene-d_6. A broadened doublet (12 Hz wide at half peak height) at δ1.85 was strongly correlated to one proton at δ2.10 and weakly correlated to three others at δ3.71, 1.58 and 1.00. The weak correlation to δ3.71 was interpretated as being the result of a nearly 90° dihedral angle between these protons, and the chemical shift of this proton required its placement both allylic and adjacent to the carboxylic acid functionality. Since the δ2.10 and 1.58 protons formed a geminal pair (from a 1-bond HETCOSY experiment, Table 1), the weaker correlation between δ1.85 and 1.58 was interpretated to also indicate a nearly 90° angle between these two protons. These geminal protons at δ2.10 and 1.58 were also correlated to two other protons, which by chemical shift (δ2.15 and 2.05) were allylic. As it was impossible to join the δ1.85 methine proton vicinally to the secondary methyl at δ1.00 without negating partial structure **B**, this third weak correlation was interpretated as a long-range (W-coupling) interaction.

The joining together of these three partial structures was accomplished by consideration of the data generated from the COSY and long-range HETCOSY experiments [15]. This latter experiment also served to confirm partial structures **A**, **B** and **C**. Partial structures **B** and **C** could be joined by their respective methine-bearing carbons as correlations were observed between the carbon (δ50.37, C-3) possessing the δ1.85 methine proton (H-3) and the methyl protons at δ1.00 (H-17), the methine proton at δ2.30 (H-10), the methylene protons at δ1.32 (H-11) and the methine proton at δ3.71 (H-2). The absence of coupling between the vicinal methines at δ1.85 (H-3) and 2.20 (H-10) indicates a 90° dihedral angle between them, as has been previously observed in metabolites with the xenicane ring system [12]. Two linkages were observed between partial structures **A** and **C**. Long-range coupling between the methine proton at δ1.85 (H-3), the olefin proton at δ5.46 (H-7) and one of the methylene protons at C-8 (δ2.40, H-8β) with the carbon atom at δ41.62 (C-5) provides the connection between C-5 and C-6. The other **A** to **C** linkage between C-1 and C-2 was provided by observing long-range coupling between the acid carbon (δ181.12, C-18) and protons at δ2.21 (H-19), 3.71 (H-2) and 1.85 (H-3), the deshielded methine carbon at δ48.02 (C-2) and protons at δ5.46 (H-9), 1.85 (H-3) and 1.58 (H-4α), as well as several other couplings across the C-1–C-2 bond (Table 1).

The *E*-geometry of the C-6–C-7 olefin was predicted on the basis of the ^{13}C NMR chemical shift for the associated methyl group (δ16.87, C-20), which compares favourably with previous assignments in this skeletal class [12]. The *Z*-stereochemistry of the C-9–C-10 olefin is therefore obliged in order to form a reasonably unstrained structure and is further indicated by a more deshielded chemical shift for the associated methyl group (δ22.43, C-19). The relative stereochemistries at C-2 and C-3 were deduced from an analysis of the coupling constants in conjunction with predictions from a Drieding model. A nearly 90° angle could be formed between the protons on these two carbons when a model was placed in a 'chaise-longue' conformation (C-1, C-9, C-8 form the back; C-2, C-3, C-7, C-6 form the seat; and C-4, C-5 the legs) with the carboxylic acid and side-chain substituents disposed to the same side (Fig. 1). This conformation and relative stereochemistry accurately predict the small coupling constant observed between the C-3 proton and the alpha-faced proton at C-4.

Dilophic acid induced an 8 mm zone of inhibition in colonies of *Bacillus subtilis* at 100 μg/ml. However, no activity was observed using the same dose against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* or *Candida albicans*. Toxicity was observed in the common goldfish (*Carassius auratus*) at a dose of 50 μg/ml, with loss of righting reflex used as the criterion for toxicity assessment.

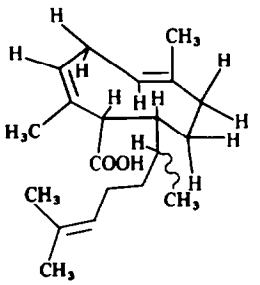


Fig. 1. Chaise-longue conformation of the 9-membered ring in dilophic acid (2).

EXPERIMENTAL

Low-field ^{1}H NMR spectra were recorded on Varian EM 360 and FT 80 spectrometers while high-field spectra were obtained on a Bruker AM 400 spectrometer. Low- and high-resolution MS were obtained through the service provided by the Environmental Health Sciences Department at Oregon State University on Finnigan 4000 and Kratos MS-50 spectrometers, respectively.

Collection and extraction. The algae were collected by hand in March 1985 from a shallow reef (−0.2 to −1.0 m) exposed to considerable wave action at Vega Baja, Puerto Rico. The fresh seaweed was immediately immersed in MeOH and stored in a freezer. Voucher specimens were prepared from this preserved material and have been deposited at the herbarium of the Department of Marine Science at the University of Puerto Rico at Mayaquez.

The seaweed was filtered from MeOH and homogenized in $CHCl_3$–MeOH (2:1) and re-extracted twice and the pooled, reduced extracts repetitively partitioned (3 ×) between H_2O and $CHCl_3$. The dried ($MgSO_4$) $CHCl_3$ layer was filtered and reduced *in vacuo* to yield 13.0 g of a dark-green viscous oil (marc 300 g).

Chromatography of the oil over a TLC grade silica gel vacuum column (9.5 cm diameter × 5 cm high) using a gradient of isooctane with increasing percentages of EtOAc yielded 23 fractions. Dictyol E (1) was isolated by thick TLC of the fraction eluting with 10% EtOAc–isooctane and identified from its ^{1}H NMR spectrum [14]. Dilophic acid (2) eluted from the vacuum column with 4% EtOAc–isooctane and was purified by prep. HPLC (10% EtOAc in isooctane, Alltech RSIL silica 10 μm, 10 mm × 50 cm) to a colourless oil (975 mg, 7.5% of organic extract). For 2: $[\alpha]_D^{25} - 116°$ (*c* 2.35, $CHCl_3$); IR ν^{CCl_4} cm^{-1}: 3400, 1715, 1460, 1425, 1288, 1230, 1200, 1120, 1070, 960, 875; UV λ_{max}^{MeOH} nm: 228, (ε 440); ^{1}H NMR (400 MHz, $CDCl_3$): δ11.8 (1H, *br s*, COOH), 5.47 (1H, *br dd*, *J* = 8.7, 1.5 Hz, H-7), 5.33 (1H, *br d*, *J* = 10.8 Hz, H-9), 5.10 (1H, *tq*, *J* = 6.4, 1.3 Hz, H-13), 3.46 (1H, *br s*, H-2), 2.96 (1H, *br ddq*, *J* = 13.6, 13.6, 2.1 Hz, H-8α), 2.44 (1H, *br dd*, *J* = 13.6, 9.9 Hz, H-8β), 2.24 (1H, *m*, H-4), 1.96–2.04 (5H, *m*), 1.93 (3H, *s*, H-20), 1.78 (3H, *s*, H-19), 1.68 (3H, *s*, H-15), 1.67 (1H, *m*, H-3), 1.60 (3H, *s*, H-16), 1.58 (1H, *m*, H-4), 1.22 (2H, *m*, H-11), 0.76 (3H, *d*, *J* = 6.6 Hz, H-17); ^{13}C NMR ($CDCl_3$, 100 MHz): δ180.34 (*q*C, C-18), 140.62 (*q*C, C-1), 135.12 (*q*C, C-6), 131.44 (*q*C, C-14), 126.93 (CH, C-7), 126.06 (CH, C-9), 124.70 (CH, C-13), 49.93 (CH, C-3), 47.62 (CH, C-2), 41.25 (CH_2, C-5), 38.11 (CH_2, C-11), 33.80 (CH_2, C-10), 29.05 (CH_2, C-4), 27.65 (CH_2, C-8), 25.95 (CH_2, C-12), 25.68 (CH_3, C-15), 22.05 (CH_3, C-19), 17.65 (CH_3, C-16), 16.81 (CH_3, C-20), 15.16 (CH_3, C-17); LR EIMS (70 eV), *m/z* (rel. int.): obs. $[M]^+$ 304 (15), 222 (12), 149 (11), 147 (17), 137 (20), 69 (100); HR EIMS (70 eV) *m/z*: obs. $[M]^+$ 304.2408 (≤ 0.1%, 0.0005 amu dev.). Elemental analysis (Galbraith) found: C, 78.70; H, 10.84. Requires; C, 78.90; H, 10.59%.

Dilophic acid methyl ester 3 (CH_2N_2) was purified by HPLC (20% EtOAc–isooctane, Alltech RSIL silica 10 μm, 10 mm × 50 cm) to give *ca* 20 mg of pure methyl ester 3 (64% yield) as an oil: IR ν^{CHCl_3} cm^{-1}: 2940, 2850, 1770, 1490, 1430, 1410, 1200, 1105, 1070, 910 cm^{-1}; ^{1}H NMR ($CDCl_3$, 80 MHz): δ5.40 (2H, *m*), 5.10 (1H, *br t*, *J* = 6.5 Hz), 3.60 (3H, *s*), 3.42 (1H, *br s*), 1.5–3.0 (10H, *m*), 1.91 (3H, *s*), 1.76 (3H, *s*), 1.67 (3H, *s*), 1.60 (3H, *s*), 1.25 (2H, *m*), 0.62 (3H, *d*, *J* = 5.5 Hz); (benzene-d_6, 400 MHz): δ5.40 (2H, *m*, H-7, H-9), 5.21 (1H, *tq*, *J* = 7.0, 1.2 Hz, H-13), 3.58 (1H, *br s*, H-2), 3.27 (3H, *s*, –OMe), 2.90 (1H, *ddq*, *J* = 13.0, 13.0, 2.1 Hz, H-8α), 2.33 (1H, *br dd*, *J* = 14.0, 8.8 Hz, H-8β), 2.26 (1H, *tq*, *J* = 6.8, 6.8 Hz, H-10), 2.23 (3H, *br s*, H-19), 2.15 (1H, *m*, H-4β), 2.06 (2H, *dt*, *J* = 7.0, 7.0 Hz, H-12), 1.95 (2H, *m*, H-5), 1.75 (3H, *s*, H-20), 1.72 (1H, *m*, H-3), 1.68 (3H, *s*, H-15), 1.58 (3H, *s*, H-16), 1.48 (1H, *m*, H-4α), 1.26 (2H, *dt*, *J* = 6.8, 7.0 Hz, H-11), 0.74 (3H, *d*, *J* = 6.8 Hz, H-17).

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