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AVRAINVILLEOL, A BROMINATED DIPHENYLMETHANE DERIVATIVE WITH FEEDING DETERRENT PROPERTIES FROM THE TROPICAL GREEN ALGA *AVRAINVILLEA LONGICAULIS**

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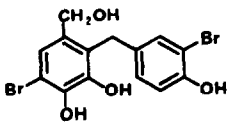
Key Word Index—*Avrainvillea longicaulis*; Udoteaceae; Chlorophyta; algal feeding deterrents; brominated diphenylmethanes.

Abstract—A new brominated diphenylmethane derivative, avrainvilleol, has been isolated from the green alga *Avrainvillea longicaulis*, and its structure determined by chemical and spectral methods. Avrainvilleol was toxic toward reef fishes at 10 μg/ml levels, showed antibacterial activity and at 800 ppm induced significant feeding deterrence in the tropical damselfish *Pomacentrus coeruleus*.

INTRODUCTION

Several previous investigations of the marine tropical green algae of the families Caulerpaceae and Udoteaceae† have shown that these seaweeds produce mainly linear sesquiterpenoids and diterpenoids, many of which possess the unique terminal *E,E*-1,4-diacetoxybutadiene group [1–5]. In connection with our interest in the chemical adaptations of these algae for defence against the abundant predators in tropical ecosystems, we have illustrated significant toxicity and feeding deterrence properties for these molecules [4, 5]. In this report, we wish to report the results of our chemical investigation of *Avrainvillea longicaulis* (Kuetzing) Murray and Boodle (Udoteaceae, Chlorophyta), an abundant shallow-water alga in the Caribbean Sea. *A. longicaulis* does not produce linear terpenoids, but instead we have isolated a brominated diphenylmethane derivative for which we suggest the name avrainvilleol and structure **1**.



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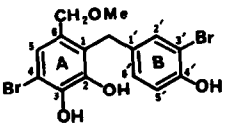

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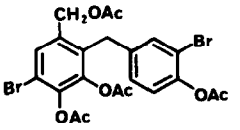
RESULTS AND DISCUSSION

Avrainvilleol (**1**) was the major metabolite (1% dry wt, 50% extract) isolated by chromatography when freshly collected *A. longicaulis* was immediately extracted with ether. When the algae were preserved in methanol and later extracted with chloroform–methanol mixtures, the corresponding methyl derivative **2** was obtained.

The structure of avrainvilleol (**1**) was assigned based upon spectral analysis and by conversion to several key derivatives. A molecular formula of $C_{14}H_{12}O_4Br_2$ was established for **1** by high resolution mass spectrometry. The 14 carbon atoms in **1** were found to be composed of 12 aromatic carbons and two non-aromatic methylenes (δ 29.9*t*, δ 67.5*t*) by ^{13}C NMR spectroscopy (Experimental). These data, taken in conjunction with UV absorption at 288 nm (ε 4400), allowed the assumption that **1** was a substituted diphenylmethane derivative.



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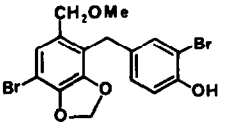

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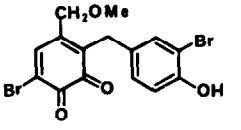

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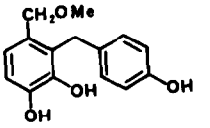

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Treatment of **1** with acidic methanol yielded **2** in quantitative yield, which reinforced our conclusion that **2** was an artefact of methanol extraction. Acetylation (acetic anhydride–pyridine) yielded a tetra-acetate ester, **3**, which possessed three phenol acetates, and one hydroxymethyl

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†Previously, we had considered green algae of the genera *Rhipocephalus* and *Udotea* to be classified within the family Codiaceae. Current taxonomic trends indicate these species to be best classified within the family Udoteaceae. We thank Professor John West and Ms. Jeanine Stojkovich, University of California, Berkeley, for their advice on this matter.

acetate. This latter assignment was recognized by comparison of the ^{1}H NMR spectra of **1** with **3**. The two-proton methylene signal in the spectrum of **1** at δ 4.50 was shifted to δ 5.00 in that of **3**, thus confirming the presence of the hydroxymethyl group.

Further examination of the 220 MHz ^{1}H NMR spectrum of **1** illustrated that one aromatic ring was penta-substituted (δ 7.11, 1H, *s*) and that the other was 1,2,4-trisubstituted (δ 6.82, 1H, *d*, J = 8 Hz; δ 6.95, 1H, *dd*, J = 8, 2 Hz; δ 7.23, 1H, *d*, J = 2 Hz). Data to locate the hydroxymethyl group, three aromatic hydroxyls and two bromine atoms on each of these rings were not readily available without further experimentation.

Treatment of the methyl derivative **2** with methylene iodide and potassium carbonate in dry acetone yielded a methylene-dioxy derivative assigned as **4**, which illustrated that **2** and hence **1** were *ortho*-hydroquinones. This latter assignment was confirmed by silver oxide oxidation of **2** to yield the corresponding bright red *ortho*-quinone, **5**.

To finally establish the sites of substitution for bromine and hence for all other substituents, the methyl derivative **2** was dehalogenated by treatment with lithium in liquid ammonia to yield the triol **6**. The ^{1}H NMR spectrum of **6** consisted of an aromatic *ortho*-coupled A_2B_2 pattern (δ 6.71, 2H, *d*, J = 8 Hz and δ 7.04, 2H, *d*, J = 8 Hz) and an aromatic *ortho*-coupled AB pattern (δ 6.73, 1H, *d*, J = 8 Hz and δ 6.81, 1H, *d*, J = 8 Hz). This result illustrated that each ring possessed one bromine atom in compounds **1** and **2**.

The conclusive structure assignment of **1** was accomplished by spin-decoupling experiments with avrainvilleol (**1**) and by a comparison of the measured ^{1}H and ^{13}C NMR shifts of **1** and **6** with predicted values. Irradiation of the hydroxymethyl group in **1** at δ 4.50 caused a marked sharpening of the aromatic proton at δ 7.11, thus placing the lone proton in ring A *ortho* to the hydroxymethyl group. Since debromination produced an AB pattern in this ring (in **6**), the proton must also be flanked by bromine. Since this ring must also possess the *ortho*-hydroquinone functionality, the substitution pattern must be as drawn in **1**. For ring B, a hydroxyl substituent must be placed at the 4′-position to generate the A_2B_2 pattern found for the ring B protons in **6**. Based upon ring activation and directing effects, bromine was initially placed *ortho* to the hydroxyl group. This was confirmed by spin-decoupling experiments with compound **1**. Irradiation of the bis-aromatic methylene at δ 3.96 produced pronounced sharpening of two protons only of ring B, observed at δ 6.95 and 7.23 and were assigned, therefore, to the 2′ and 6′-positions. This latter observation also confirmed the substitution pattern defined earlier for ring A.

Calculations of the predicted ^{13}C NMR bands for **1** [7] were also compared with those observed and found to be in good agreement (Experimental). The calculated and predicted ^{1}H NMR shifts for **6** were also in good agreement with predicted values (Experimental).

Using bioassays previously described [4, 6], avrainvilleol was found to be toxic toward reef fishes at the 10 μg/ml level. At 800 ppm **1** induced significant feeding avoidance behavior in the herbivorous damselfish, *Pomacentrus coeruleus*. Using standard agar plate-assay disc methods, avrainvilleol showed moderate to strong antibacterial activity toward the pathogenic marine bacterium *Vibrio anguillarum* (100 μg/disc levels).

While the isolation of brominated diphenylmethane derivatives has not been reported from members of the green algae (Chlorophyta), metabolites similar to avrainvilleol have been isolated from the marine annelid *Thelepus setosus* [8] and from the red seaweeds *Rhodomela confervoides* [9] and *Rytiphlea tinctoria* [10]. Compounds of this general type appear to be the products of dimerization of more ubiquitous precursors such as halo-*p*-hydroxybenzyl alcohol derivatives.

EXPERIMENTAL

Isolation of avrainvilleol (**1**) *and its methyl derivative* (**2**). Freshly collected *A. longicaulis*, Carrie Bow Cay, Belize, May 1978, was ground in a hand-operated grinder and the resultant slurry was repeatedly extracted with Et_2O. The combined extracts were dried and reduced to a dark green tar. CC of the extract with C_6H_6 on Bio-beads SX-8 yielded avrainvilleol (**1**) as a viscous oil (1% dry wt algae, 50% Et_2O extract). Avrainvilleol showed the following spectral features: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3300, 2950, 1600, 1575, 1480, 1270; UV λ_{max}^{MeOH} nm: 288 (ε = 4400); ^{1}H NMR (220 MHz, $CDCl_3$): δ 3.96 (2H, *s*), 4.50 (2H, *s*), 6.82 (1H, *d*, J = 8 Hz), 6.95 (1H, *dd*, J = 8, 2 Hz), 7.11 (1H, *s*), 7.23 (1H, *d*, J = 2 Hz); ^{13}C NMR ($CDCl_3$): δ 29.9 (*t*), 67.5 (*t*), 108.3 (*s*), 109.8 (*s*), 116.2 (*d*), 122.8 (*d*), 125.1 (*s*), 128.5 (*d*), 132.1 (*d*), 133.1 (*s*), 133.5 (*s*), 141.0 (*s*), 144.3 (*s*), 151.6 (*s*); MS *m/z*: 406, 404, 402 $[M]^+$, 400, 388, 383, 331, 299, 261, 242, 232, 230, 227, 226, 216, 214, 197, 185, 139, 115, 105; high resolution mass measurement for $C_{14}H_{12}O_4Br_2^{79}$: 401.9072 (calc. 401.9102); calculated ^{13}C NMR values [7] (aromatic carbons only): 109.8 (*s*), 110.3 (*s*), 117.5 (*d*), 122.9 (*s*), 125.0 (*d*), 124.0 (*s*), 128.4 (*d*), 133.5 (*d*), 136.3 (*s*), 144.4 (*s*), 145.1 (*s*), 158.9 (*s*). Extraction of *A. longicaulis*, collected at the same time, but preserved in MeOH, followed by chromatography as described above, yielded exclusively the methyl derivative **2** which illustrated the following spectral features: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3220, 3020, 2970, 1600, 1575, 1480, 1270, 1090, 1025; ^{1}H NMR (220 MHz, $CHCl_3$): δ 3.34 (3H, *s*), 3.98 (2H, *s*), 4.29 (2H, *s*), 6.88 (1H, *d*, J = 8 Hz), 7.02 (1H, *dd*, J = 8, 2 Hz), 7.05 (1H, *s*), 7.24 (1H, *d*, J = 2 Hz); ^{13}C NMR (20 MHz, $CDCl_3$): δ 30.1 (*t*), 57.9 (*q*), 72.2 (*t*), 108.0 (*s*), 109.8 (*s*), 116.1 (*d*), 124.0 (*d*), 125.9 (*s*), 128.5 (*d*), 130.1 (*s*), 132.1 (*d*), 133.2 (*s*), 141.4 (*s*), 144.3 (*s*), 151.3 (*s*); MS *m/z*: 307, 305 $[M - MeOH - Br]^+$, 261, 259, 226, 197, 191, 149, 113, 91, 81, 69, 55, 43, 41. To confirm the relationship of **1** and **2** through solvolysis, avrainvilleol was treated with traces of *p*-toluenesulfonic acid in MeOH. These conditions converted **1** to **2** in quantitative yield.

Avrainvilleol tetra-acetate (**3**). 10 mg avrainvilleol (**1**) were combined with 2 ml Ac_2O and 2 ml dry pyridine at room temp. and the soln was stirred overnight. The mixture was then hydrolysed and next partitioned between Et_2O and H_2O. The Et_2O phase was dried ($MgSO_4$), filtered, and reduced under vacuum to yield the corresponding tetra-acetate, **3**, which illustrated the following spectral features: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2900, 1770, 1740, 1600, 1575, 1380, 1245, 1175, 1045, 1020, 910; ^{1}H NMR (220 MHz, $CDCl_3$): δ 1.94 (3H, *s*), 2.18 (3H, *s*), 2.32 (6H, *s*), 3.94 (2H, *s*), 5.00 (2H, *s*), 6.99 (2H, *s*), 7.23 (1H, *s*), 7.59 (1H, *s*); MS *m/z*: 509 $[M - 59 (-OAc)]^+$.

Methylene-dioxy derivative (**4**). A soln of 25 mg of the Me derivative, **2**, and excess Na_2CO_3 were combined with 10 ml dry Me_2CO and cooled to 0°. A soln of excess CH_2I_2 in 2 ml dry Me_2CO was slowly added and the soln was stirred for 20 min and allowed to warm to room temp. over a 2 hr period. The reaction mixture was partitioned between Et_2O and H_2O and the combined Et_2O phases were dried ($MgSO_4$) and reduced under vacuum. The mixture obtained was subjected to prep. TLC (Si gel) to yield the methylene-dioxy derivative, **4** (16 mg, 61%), as

a viscous oil which showed the following spectral features: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 2940, 1600, 1480, 1430, 1225, 1070, 945; ^1H NMR (220 MHz, $CDCl_3$): δ 3.30 (3H, *s*), 3.91 (2H, *s*), 6.03 (2H, *s*), 6.98 (1H, *s*), 7.06 (1H, *dd*, J = 8, 2 Hz), 7.19 (1H, *d*, J = 8 Hz), 7.34 (1H, *d*, J = 2 Hz).

Ortho-*quinone derivative* (**5**). The Me derivative **2** (15 mg) was dissolved is 5 ml dry MeOH and excess freshly prepared Ag_2O was added with stirring. After 30 min the insoluble reagent was filtered and the solvent was removed under vacuum to yield a dark red glass. Purification via prep. TLC yielded the *ortho*-quinone as a red viscous oil which displayed the following spectral features: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 2940, 1670, 1480, 1350, 1175, 1100; ^1H NMR (220 MHz, $CDCl_3$): δ 3.41 (3H, *s*), 3.65 (2H, *s*), 4.28 (2H, *s*), 5.91 (1H, OH), 6.90 (1H, *d*, J = 8 Hz), 6.98 (1H, *d*, J = 8 Hz), 7.23 (1H, *s*), 7.71 (1H, *s*); UV λ_{max}^{MeOH} nm: 287 (ε = 4000); MS: *m/z* 418, 416, 414 $[M]^+$, 387, 385, 383 $[M - OMe]^+$, 307, 305 $[M - OCH_2 - Br]^+$.

Dehalogenation of the methyl derivative **2**. 2 cm Li wire was chopped into pieces and added to 50 ml dry NH_3 maintained at $-78°$. The soln was stirred for 10 min in which time a dark blue colour resulted from complete dissolution of the Li. The Me derivative **2** (50 mg) was added using a syringe in 2 ml dry Et_2O and the soln was stirred for 30 min, then quenched with cautious addition of solid NH_4Cl. The reaction was allowed to warm to room temp. and the residue was partitioned between Et_2O and H_2O. The combined Et_2O phases were dried ($MgSO_4$) and evaporated to yield a mixture composed mainly of the dehalo derivative **6**. Reverse-phase HPLC (μ-C_{18}, 25% H_2O–MeOH) yielded the triol **6** as a viscous oil which illustrated the following spectral features: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400; ^1H NMR (220 MHz, $CDCl_3$): δ 3.33 (3H, *s*), 4.03 (2H, *s*), 4.36 (2H, *s*), 6.72 (2H, *d*, J = 8 Hz), 6.73 (1H, *d*, J = 8 Hz), 6.81 (1H, *d*, J = 8 Hz), 7.04 (2H, *d*, J = 8 Hz); HRMS: $[M]^+$ *m/z* 260.1027 ($C_{15}H_{16}O_4$), *m/z* 228.0780 ($C_{14}H_{12}O_3$, $[M^+ - MeOH]^+$).

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