Journal of Natural Products
Vol. 50, No. 3, pp. 368-374, May-Jun 1987

5′-HYDROXYISOAVRAINVILLEOL, A NEW DIPHENYLMETHANE DERIVATIVE FROM THE TROPICAL GREEN ALGA *AVRAINVILLEA NIGRICANS*

MARCELO COLON, PABLO GUEVARA, WILLIAM H. GERWICK*, [1]

Department of Chemistry, University of Puerto Rico Rio Piedras, Puerto Rico 00931 and College of Pharmacy, Oregon State University, Corvallis, Oregon 97331

and DAVID BALLANTINE

Department of Marine Sciences, University of Puerto Rico Mayaguez, Puerto Rico 00708

ABSTRACT.—A new brominated diphenylmethane, 5′-hydroxyisoavrainvilleol, was isolated from the tropical green macrophyte, *Avrainvillea nigricans*, using conventional chromatographic techniques. The structure is based on a combination of spectrochemical arguments, including a detailed analysis of the long range ^{1}H-^{13}C coupling constants. The natural product shows gram-positive antimicrobial activity.

While the incidence of bromoperoxidase activity is greatest among species of red algae (Rhodophyta), significant numbers of green algae (Chlorophyta) also show this activity (1). Furthermore, bromoperoxidase activity reaches maximum levels in some species of green algae (1), and the enzymes responsible for this activity have been isolated and characterized in several cases (2,3). In this light, the isolation of only a few halogenated natural products from the Chlorophyta is surprising; in fact, these seaweeds as a group have been a richer source of new oxygenated terpenes (4).

To explore the potential of green algae to produce halogenated secondary metabolites in the context of our exploration of Puerto Rican seaweeds as a source of new biomedicinals, we have investigated the natural products of the tropical green alga *Avrainvillea nigricans* Decaisne (Udoteaceae). In addition to finding halogenated metabolites previously reported from another species of *Avrainvillea*, *A. longicaulis* (5), as well as from Rhodophyta species (6-8), we report here a new diphenylmethane derivative containing two bromine atoms and possessing moderate antibiotic activity against several human pathogens.

EXPERIMENTAL

INSTRUMENTATION.—Ir spectra were recorded on a Beckman AccuLab 7 and uv spectra on an Aminco DW-2a UV-Vis Spectrometer. Low resolution ^{1}H-nmr spectra were obtained on Varian T-60, EM-360, FT-80a, and JEOL FX90Q spectrometers, while high field ^{1}H- and ^{13}C-nmr spectra were obtained on a Bruker AM-400 spectrometer. Low resolution mass spectra were obtained on Varian Mat CH7 and Finnigan 4000 spectrometers, the latter fitted with a model 4500 ion source. All solvents were distilled from glass prior to use.

COLLECTION, EXTRACTION AND ISOLATION.—*A. nigricans* was collected by hand using snorkeling equipment in a small bay at a depth of 1.5-3.5 m on the seaward side of Isla Guayacan off the southwest coast of Puerto Rico in June 1984. Voucher material has been deposited at the herbarium of the Department of Marine Sciences, University of Puerto Rico. Fresh *A. nigricans* was frozen shortly after collection and maintained frozen until workup. A total of 1.3 kg (dry wt) of the alga was homogenized in distilled H_2O and then repetitively extracted with Et_2O and EtOAc to yield 3.1 g lipid extract. Test extractions of small portions of the alga showed degradation of some of the natural products in the presence of $CHCl_3$. Initial fractionation using vacuum chromatography over Si gel with a stepwise gradient of hexane/CH_2Cl_2/EtOAc, followed by flash chromatography over Si gel (hexane/EtOAc gradient), gave sequentially the two known compounds, avrainvilleol [**1**], (270 mg, 8.7% of extractables) (5) and 3-bromo-4,5-dihydroxyben-

[1]Address all correspondence to: College of Pharmacy, Oregon State University, Corvallis, Oregon 97331.

zyl alcohol [**2**] (451 mg, 14.5% of extractables) (6-8), and, as the most polar natural product, a new compound [**3**]. Final purification of compound **3** using preparative thick layer chromatography afforded a relatively small quantity (48 mg, 1.5% of extractables) of a highly unstable, light yellow oil.

3-Bromo-4,5-dihydroxybenzyl alcohol [**2**].—Compound **2** showed the following: ^{1}H nmr (Me_2CO-d_6, 90 MHz) δ 7.06 (1H, d, J=2.0 Hz), 6.94 (1H, d, J=2.0), 6.85 (2H, bs, D_2O exchangeable), 4.59 (2H, bs); Acetate **5**: ^{1}H nmr (Me_2CO-d_6, 60 MHz) δ 7.65 (1H, d, J=2.0 Hz), 7.33 (1H, d, J=2.0 Hz), 5.10 (2H, bs), 2.35 (3H, s), 2.30 (3H, s), 2.10 (3H, s); eims as in Kurata and Amiya (8).

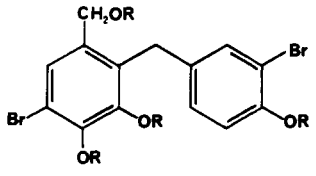
5'-Hydroxyisoavrainvilleol [**3**].—Compound **3** uv λ max (MeOH) 261 nm (ε=2,440); ir ν max ($CHCl_3$) 3250, 2950, 1610, 1570, 1490, 1425, 1350, 1280, 1170, 1080, 980, 840 cm^{-1}; ^{1}H nmr (90 MHz, Me_2CO-d_6) δ 7.04 (1H, s, H-5), 6.69 (1H, d, J=2.0, H-2'), 6.50 (1H, d, J=2.0, H-6'), 4.46 (2H, bs, H-6a), 4.06 (2H, bs, H-1a); eims *m/z* (rel. int.) 422 (8.9), 420 (18.5), 418 ($C_{14}H_{12}{}^{79}Br_2O_5$, 10.9), 404 (19.2), 402 (19.0), 385 (11.7), 323 (22.3), 322 (12.7), 321 (20.2), 305 (10.6), 277 (8.3), 275 (7.6), 244 (26.6), 243 (15.2), 242 (40.2), 232 (10.8), 230 (13.2), 229 (16.2), 213 (14.1), 203 (7.6), 201 (8.1), 197 (7.6), 139 (12.9), 121 (18.1), 110 (10.0), 82 (100.0), 81 (39.2), 80 (97.6), 79 (38.6), 77 (11.1), 65 (10.0), 58 (47.2), 57 (13.5), 55 (15.2); cims (negative ion, CH_4) 422 (6.8), 420 (20), 418 (8.4),402 (420-H_2O, 13.6), 339 (418-Br, 100), 321 (418-Br, H_2O 11.3), 258 (418-2HBr, 57.2), 242 (418-2Br, H_2O, 32.5).

Acetylation of 5'-hydroxyisoavrainvilleol [**3**].—To 5.4 mg (25 μmoles) of compound **3** was added 0.5 ml of pyridine and 0.5 ml of Ac_2O at rt with stirring. After 21 h the reaction mixture was diluted with Et_2O, quenched with ice, washed three times with 5% HCl, three times with saturated $NaHCO_3$, and once with H_2O, dried (anhydrous $MgSO_4$), and evaporated in vacuo to give 3.9 mg of product (6.2 μmoles, 25%). This material was pure by tlc and ^{1}H-nmr analysis. Ir ν max ($CHCl_3$) 2970, 1770, 1720, 1600, 1570, 1370, 1250, 1170, 1080, 1050, 890, 880 cm^{-1}; ^{1}H nmr (400 MHz, Me_2CO-d_6) δ 7.43 (1H, s), 7.22 (1H, d, J=2), 6.95 (1H, d, J=2), 5.12 (2H, bs), 4.37 (2H, bs), 2.35 (3H, s), 2.29 (3H, s), 2.22 (3H, s), 2.18 (3H, s), 1.82 (3H, s).

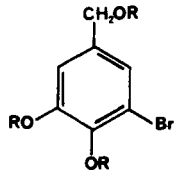
RESULTS AND DISCUSSION

Tlc examination of lipid extracts of *A. nigricans* and *A. longicaulis* from similar habitats in southern Puerto Rico showed that these two species both contain one distinctive compound in common (0.38 Rf in 60% EtOAc/hexane on Si gel, uv absorbance at 256 nm and grey char with H_2SO_4 and heating). Repeated flash chromatography of the lipid extract from a larger scale collection of *A. nigricans* led to the isolation of this major compound, and it was identified via comparison with published data as avrainvilleol [**1**] (5). Although this was the only major metabolite present in the *A. longicaulis* extract, two related but less abundant compounds from the *A. nigricans* extract were also isolated by flash chromatography. The less polar and more abundant of these was the previously described natural product **2** as determined by its characteristic ^{1}H-nmr and mass spectral features (see experimental) (6-8). The more polar compound isolated from *A. nigricans*, **3**, which was present in the least concentration, had spectral features unlike any known compound within the structure classes described by **1** and **2**, and, hence, its structure elucidation was undertaken by analysis of its spectral and chemical properties.

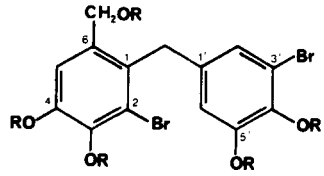
Compound **3** was a light yellow oil showing typical aromatic uv absorptions (λ max=261, ε=2440) and a very broad OH stretch in its ir spectrum (ν_{OH}=2800-3600 cm^{-1}). Furthermore, compound **3** was highly unstable and decomposed to a dark yellow tar even under N_2 at −20°. Low resolution mass spectra (ci and ei) showed a clear parent ion cluster at *m/z* 418/420/422, indicative of a dibromo compound and consistent with the molecular formula $C_{14}H_{12}O_5Br_2$. Several attempts at obtaining high resolution mass spectral data were unsuccessful; however, this molecular formula was in part confirmed from ^{13}C-nmr (14 carbon atoms observed) and ^{1}H-nmr (7 non-exchangeable protons observed) spectral analyses. Compound **3** formed a pentaacetate [**6**] upon peracetylation, thus indicating the presence of five hydroxyl groups. Summation of these various data (Br_2 from lrms, C_{14} from ^{13}C nmr, H_7 from ^{1}H nmr and 5×OH



1 R=H
4 R=Ac



2 R=H
5 R=Ac



3 R=H
6 R=Ac

from acetylation and ^{1}H-nmr analysis) yields a molecular formula of $C_{14}H_{12}O_5Br_2$, fully consistent with the observed parent ion for **3** in the lrms.

Proton bands for two broadened and deshielded methylene groups (Table 1), closely resembling those in avrainvilleol [**1**] (5), were assigned to a bisbenzylic methylene (δ 4.06) and benzylic alcohol methylene (δ 4.46). Selective homonuclear irradiations showed the bisbenzylic protons to be allylically coupled to two *meta* coupled protons at δ 6.50 and 6.69 (Table 1). The aromatic ring containing these two proton substituents was also identified as containing one bromine atom and two hydroxyl functionalities by virtue of a prominent mass spectral fragment at 201/203 (8.1:7.6, $C_7H_6O_2Br$), representing cleavage **a** (Figure 1) to yield a stabilized tropylium ion. As the two *meta* coupled protons in this ring were non-equivalent, these bromine and hydroxyl substituents could be present in only one possible substitution pattern as given in partial structure **A**.

Consideration of the molecular formula for compound **3** required the second aromatic ring to possess one bromine atom, two hydroxyl groups, a benzylic alcohol, one pro-

TABLE 1. ^{1}H- and ^{13}C-nmr Data for 5′-Hydroxyisoavrainvilleol [**3**]

Position	^{1}H/J^a	^{13}C calc^b	^{13}C obs^c	J ^{1}H-^{13}C^d	J ^{1}H-^{13}C (irr)^e
1	—	130.1	128.88	m	d, J=7.3 Hz
2	—	112.3	114.64	td, J=5.5, 2.2	d, J=2.2
3	—	145.5	142.67	brd, J=8.1	shd, J=8.1
4	—	141.2	144.79	d, J=2.8	d, J=2.8
5	7.04, bs	116.0	114.94	dm, J=157	d, J=144
6	—	137.9	133.34	tm, J=7.4	brs
1′	—	134.4	134.01	m	brs
2′	6.69, bd, J=2.0	126.7	123.36	ddt, J=161, 8, 4	dd, J=145, 6.8
3′	—	111.6	110.00	m, W½=7.5	m, W½=5.7
4′	—	143.7	141.79	ddm, J=7.5, 7.5	dd, J=6.5, 6.5
5′	—	145.8	146.54	d, J=1.7	d, J=1
6′	6.50, bd, J=2.0	117.4	115.00	dm, J=151	dd, J=136, 8.3
1a	4.06, bs	—	36.25	tt, J=138, 4.1	t, J=3.7
6a	4.46, bs	—	62.68	tm, J=145	tm, J=41.5

[a]Spectrum obtained at 90 MHz on a JEOL FX 90Q in Me_2CO-d_6. Selective homonuclear irradiations showed that the protons at carbons 5 and 6a and at 2′, 6′, and 1a formed two isolated spin systems.

[b]Calculations were made assuming additivity of substituent parameters available in Gordon and Ford (9).

[c]Approximately 8 mgs of **3** in a 5 mm tube in Me_2CO-d_6, overnight accumulation on a Bruker AM 400 spectrometer.

[d]Overnight accumulation of 4 mgs of **3** in Me_2CO-d_6 in a 5 mm tube on a Bruker AM 400 spectrometer. The exchangeable protons in **3** were first replaced with deuterium by twice dissolving the sample in 0.5 ml Me_2CO-d_6 containing 1 drop D_2O followed by evaporation in vacuo. The broad band proton decoupler was gated between carbon accumulations to gain nOe enhancements of these signals.

[e]The sample identified in d was run under identical conditions as those described in d except that an additional selective ^{1}H decoupling pulse at δ 4.06 was introduced during the carbon accumulation. Some spill-over of proton irradiation power reduced the magnitude of most coupling constants.

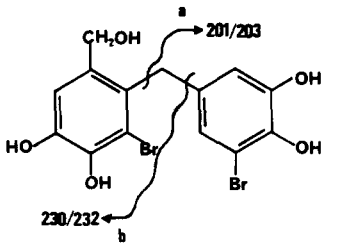
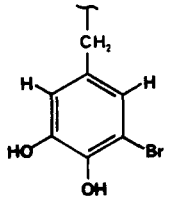


FIGURE 1. Prominent mass spectral cleavages of 5′-hydroxyisoavrainvilleol [3]



partial structure **A**

ton, and partial structure **A** as substituents. These substituents were confirmed by mass spectral fragments at 230/232 (13.2:10.8, $C_8H_7O_3Br$) resulting from cleavage **b** (Figure 1) again to produce a stabilized tropylium ion. The placement of the one aromatic proton in ring A was secured by observing allylic coupling between it and the benzylic alcohol methylene protons. As these substituents are analogous to those found in the A-ring of avrainvilleol [**1**] (5), a similar substitution pattern was at first hypothesized for compound **3**. However, this possibility was dismissed due to large variations in the calculated (9) versus measured ^{13}C-nmr chemical shifts for the C-2 carbon (over 10 ppm error). The best fit between calculated and measured ^{13}C-nmr shifts for the A-ring carbons was obtained when the order of hydroxyl and bromine substituents was reversed relative to avrainvilleol [**1**] (Table 1). These carbon assignments are in close agreement to those of a similar diphenylmethane which has related substituents in the same substitution pattern. This pattern was further supported by observing the effect of peracetylation of compound **3** on the chemical shift of the lone proton substituent in this ring. The behavior of this proton in the model system afforded by peracetylation of compound **2** was first explored, and a good correlation between observed and calculated differences in chemical shift upon acetylation was made (Table 2). The shift of 0.39 ppm in the A-ring aromatic proton upon acetylation of compound **3** to **6** argues favorably for hydroxyl functionalities positioned *ortho* and *meta* to this proton.

Proof of the substitution pattern in the A-ring was accomplished by showing the existence of a 3-bond coupling constant (5.5 Hz) between the bisbenzylic methylene protons (H-1a) and the carbon bearing the bromine (C-2) in the A-ring (Table 1). This carbon shift assignment was based on careful comparisons of its unique shift with that calculated (9) and obtained from model compounds (5,10). Further proof of this substitution pattern was obtained by comparing predicted (9) and observed long range ^{1}H-^{13}C coupling constants for various of the carbon atoms in metabolite **3** (Table 1).

Biogenetically, the isolation of compound **2** from this seaweed helps strengthen the hypothesis that the origin of these diphenylmethanes is from shikimic acid pathway via tyrosine (11). Dimerization of compound **2** via condensation to the hydroxyl side of one monomer would lead to the substitution pattern observed in avrainvilleol [**1**] while

TABLE 2. Calculated and Observed Effects of Acetylation Upon ^{1}H-nmr Chemical Shifts in Compounds **2** and **3**[a]

Compounds compared	position	shift for R=H[b]	shift for R=Ac[b]	difference[c]
2 [5]	H-2 calculated	6.80	7.32	0.52
2 [5]	H-2 observed	7.06	7.65	0.59
2 [5]	H-6 calculated	6.45	6.86	0.41
2 [5]	H-6 observed	6.94	7.33	0.39
3 [6]	H-5 calculated	6.44	6.85	0.41
3 [6]	H-5 observed	7.04	7.43	0.39
3 [6]	H-2′ calculated	6.80	7.32	0.52
3 [6]	H-2′ observed	6.69	7.22	0.53
3 [6]	H-6′ calculated	6.45	6.86	0.41
3 [6]	H-6′ observed	6.50	6.95	0.45

[a]Calculations from data available in Jackman and Sternhell (12).
[b]All spectra obtained in Me_2CO-d_6.
[c]Subtraction of ^{1}H-nmr shift in hydroxy natural product from the ^{1}H nmr shift in acetoxy derivative.

reaction at the opposite side would yield 5′-hydroxyisoavrainvilleol [**3**] (Figure 2). Although it has been suggested (11) that avrainvilleol [**1**] from *A. longicaulis* may actually be a bacterial natural product from epibiotic microorganisms rather than an algal metabolite, principally on chemotaxonomic grounds, the isolation of natural products **1-3** as major compounds from *A. nigricans* implicates an algal source for these metabolites. Tlc examination of the separately extracted blade, stipe, and haptera portions of several *A. nigricans* plants clearly demonstrated that these secondary metabolites are present in greatest quantity in the blade region, suggesting a defensive role for these natural products.

Interestingly, *A. nigricans* from the western Caribbean was earlier found to be devoid of halogenated organic metabolites and bromoperoxidase activity (1). This may represent a temporal or geographic variation in secondary metabolite biosynthesis. A

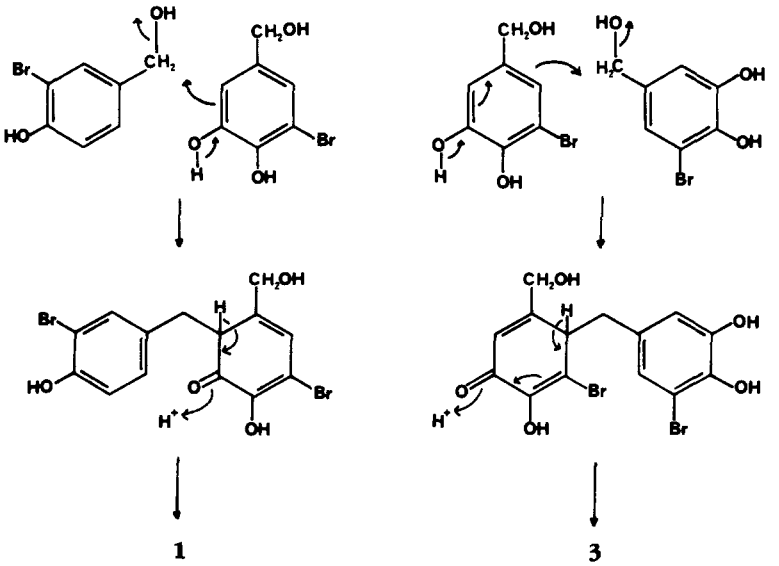


FIGURE 2. Proposed biogenetic relationship of avrainvilleol [**1**] and 5′-hydroxyavrainvilleol [**3**]

TABLE 3. Antimicrobial[a] and Cytotoxic Activities of Metabolites **1**, **2**, and **3**

Compound	*Bacillus subtilis*[b]	*Staphylococcus aureus*[b]	*Pseudomonas aeruginosa*[b]	*Escherichia coli*[b]	*Serratia marcesens*[b]	*Candida albicans*[b]	KB[c] Cells
1[d]	25 µg- 9 mm	25 µg-10 mm	Neg	Neg	200 µg- 9 mm	Neg	10-100 µg
	50 µg-12 mm	50 µg-12 mm					
	100 µg-14 mm	100 µg-16 mm					
2[e]	25 µg- 8 mm	25 µg- 8 mm	100 µg-8 mm	200 µg-10 mm	100 µg- 9 mm	200 µg-8 mm	8.9 µg
	50 µg-10 mm	50 µg-11 mm	200 µg-9 mm		200 µg-13 mm		
	100 µg-12 mm	100 µg-13 mm					
3[d]	25 µg- 9 mm		Neg	Neg	Neg	Neg	[f]
	50 µg-10 mm						
	100 µg-11 mm	100 µg-10 mm					

[a]Paper disc/agar plate antibiotic sensitivity methodology. Measurements of inhibition zones represent diameters (6.5 mm disc).

[b]Cultures of these microorganisms obtained from stock cultures in the Stock Culture Collection, Department of Microbiology, Oregon State University, Ms. I. Kaattari, curator.

[c]Values given indicate ID_{50} values (µg/ml).

[d]Neg indicates no activity at 100 µg/disc.

[e]Neg indicates no activity at 200 µg/disc.

[f]Untested.

similar variation has been observed in secondary metabolite biosynthesis between *A. longicaulus* from the Caribbean and the western Pacific (11).

The three *A. nigricans* natural products [**1-3**] were all inactive in our goldfish toxicity assay at a test level of 10 μg/ml. While all of these compounds show some inhibitory activity to gram positive microorganisms, the "monomer" **2** was the strongest and also showed the broadest spectrum of activity (Table 3). Only the monomer [**2**] showed significant cytotoxicity to KB cells in tissue culture (ED_{50}=8.9 μg/ml).

ACKNOWLEDGMENTS

We thank Dr. Adriana Baez of the Medical School at the University of Puerto Rico for running cytotoxicity assays of our compounds to KB cells. We gratefully acknowledge the efforts of Mr. Roger Kohnert for help in obtaining nmr data on the OSU Department of Chemistry's Bruker AM 400 spectrometer, purchased in part through grants from the National Science Foundation (CHE-8216190) and from the M.J. Murdock Charitable Trust. We further acknowledge the OSU College of Agricultural Chemistry's Mass Spectrometer Facility for running mass spectra. This work was supported by the Puerto Rico (MR/D-12-1) and Oregon (R/PD-47) Sea Grant Programs.

LITERATURE CITED

1. W.D. Hewson and L.P. Hager, *J. Phycol.*, **16**, 340 (1980).
2. J.A. Mantley and L.P. Hager, *J. Biol. Chem.*, 256 (1981).
3. D.G. Baden and M.D. Corbett, *Biochem. J.*, **187**, 205 (1980).
4. D.J. Faulkner, *Nat. Prod. Rep.*, 251 (1984).
5. H.H. Sun, V.J. Paul, and W. Fenical, *Phytochemistry*, **22**, 743 (1983).
6. von K.W. Glombitza, H. Stoffelen, U. Murawski, J. Bielaczek, and H. Egge, *Planta Med.*, **25**, 105 (1974).
7. M. Pedersen, P. Saenger, and L. Fries, *Phytochemistry*, **13**, 2273 (1974).
8. K. Kurata and T. Amiya, *Bull. Chem. Soc. Jpn.*, **53**, 2020 (1980).
9. A.J. Gordon and R.A. Ford, "The Chemist's Companion," John Wiley and Sons, New York, 1972, p. 285.
10. K. Kurata and T. Amiya, *Chem. Lett.*, 1435 (1977).
11. W. Fenical and V.J. Paul, *Hydrobiologia*, **116/117**, 135 (1984).
12. L.M. Jackman and S. Sternhell, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd ed., Permagon Press, Oxford, 1969, p. 202.

Received 2 September 1986