ing the ordinary unimodal (r_1) and bimodal (r_2) vector lengths to determine whether one-directional or axial clustering fits the data. The calculations are, however, not overwhelming, and compared to the improvement obtained in practical cases where the broken axis approach provides a considerably better fit to the observed data than unimodal and antipodally symmetric bimodal distributions, it seems reasonable to perform the calculations. The method may give a better understanding of the orientation behavior of living organisms, as well as in other areas of research.

A computer program for the calculations inherent in the new technique is available from the authors upon request.

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Vidalols A and B, new anti-inflammatory bromophenols from the Caribbean marine red alga *Vidalia obtusaloba*

D. F. Wiemer*, D. D. Idler and W. Fenical

Scripps Institution of Oceanography, University of California, San Diego, La Jolla (California 92093-0236, USA)
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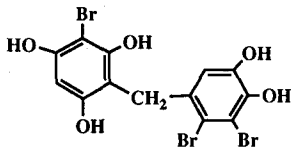
Abstract. Chemical studies of the Caribbean red alga *Vidalia obtusaloba* have resulted in the isolation of two new bromophenolic metabolites, vidalols A and B (**1, 2**). The new compounds were discovered as part of an organized effort to isolate new naturally-occurring anti-inflammatory agents with a focus upon those which may function through the inhibition of phospholipase A_2.
Key words. Anti-inflammatory; bromophenols; red algae; marine natural products.

In connection with our interest in the isolation of new anti-inflammatory compounds from marine sources [1], we have investigated the secondary metabolites of the Caribbean red alga *Vidalia obtusaloba* (Rhodophyta; Rhodomelaceae). Ship-board testing of the crude extract of this alga showed potent in vitro inhibition of bee venom-derived phospholipase A_2 (PLA_2). The subject of this report is the isolation of two new anti-inflammatory bromophenols, vidalols A (**1**) and B (**2**), which inhibit PLA_2 and effectively control edema in the phorbol ester-mouse ear assay [2].

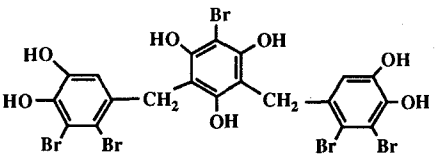
Vidalia obtusaloba (1 kg wet) was collected in shallow water near St. Anne's anchorage, Martinique, in July, 1986. The alga was freeze-dried and repeatedly extracted with 10% methanol in chloroform. After concentration of the combined extracts in vacuo, the crude residue (29 g) was fractionated by silica vacuum flash chromatography. Fractions eluted with 80% EtOAc in isooctane were further purified by gravity elution silica column chromatography to yield vidalol A (**1**, 1.235 g) and vidalol B (**2**, 54 mg) as pure, non-crystalline solids.

Vidalol A (**1**) analyzed for $C_{13}H_9O_5Br_3$ by HRFAB mass spectrometry (M^+ m/z = 481.8029). Fragment ions at m/z 279/281/283 and m/z 216/218 showed that vidalol A was easily cleaved into two benzyl cations with the compositions $C_7H_5O_2Br_2$ and $C_7H_6O_3Br$, respectively. The ^{13}C NMR spectrum of vidalol A in $CDCl_3$ showed 13 resonances, 12 of which were aromatic [3]. A single methylene resonance at 31.9 ppm indicated that vidalol A was a diphenylmethane derivative.

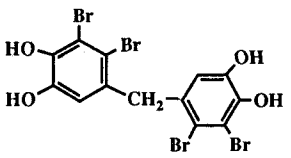
Acetylation of vidalol A with acetic anhydride in pyridine yielded a penta-acetate derivative illustrating that all five oxygen atoms are phenolic hydroxyls. The ^1H NMR spectrum of **1**, recorded in $CDCl_3$ showed two overlapping aromatic protons as broad singlets at δ 6.35 and a broadened 2 H singlet at δ 3.90. These overall data indicated that vidalol A was a diphenylmethane derivative with one ring bearing two bromine atoms and two hydroxyl groups and the other one bromine and three hydroxyl functionalities. The regiochemistry of the substituents on the dibromo-dihydroxy-benzene ring was easily established by comparison of the ^{13}C NMR fea-



1, vidalol A



2, vidalol B



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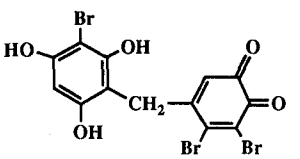

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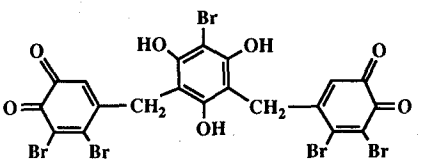

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tures for **1** with those of a related diphenylmethane derivative **3**, isolated from the red alga *Rhodomela larix* from Japan[4]. The substitution pattern of the remaining penta-substituted ring was established by comparison of the calculated verses observed ^{13}C NMR bands of the remaining aromatic carbons[5]. Especially diagnostic were the highly shielded resonances at 96.4 (d) and 90.8 (s) ppm which require a reinforcing network of ortho- and para-hydroxyl functionalities. The 3-bromo-2, 4, 6-trihydroxybenzene constellation in **1**, with the lone proton meta to the methylene carbon, is the only possible structure which satisfies this requirement. The structure of the pentamethyl ether derivative of vidalol A, obtained by X-ray analysis, was reported in 1976 as part of an investigation of the Mediterranean red alga *Rytiphlea tinctoria*[6]. This alga apparently contains vidalol A, but the natural product was not isolated nor characterized.

Vidalol B (**2**) analyzed for $C_{20}H_{13}O_7Br_5$ by HRFAB mass spectrometry (M^+ $m/z = 761.6565$ for the $^{79}Br_4{}^{81}Br$ isotope peak, calcd $m/z = 761.6558$). The ^{13}C NMR features of vidalol B were deceptively simple with only 11 resonances observed[7]. The ^1H NMR spectrum in $(CD_3)_2CO$ showed only two bands, a singlet at δ 6.33 and a broadened singlet at δ 4.00, the latter band of double intensity in relation to the former. Analysis of the ^{13}C NMR data for vidalol B again showed the presence of a 2,3-dibromo-4,5-dihydroxy benzene group[4]. Because this accounts for 7 of the 11 resonances observed, the molecule must contain two of these benzene functional groups attached to a fully substituted aromatic ring. Symmetry considerations require the remaining benzene ring to possess three hydroxyl groups and one bromine atom arranged with C_{2V} symmetry overall. Thus, structure **2** can be formulated for vidalol B.

Bromophenols of simple and more complex structures, apparently derived via the degradation of tyrosine, are common metabolites of red algae of the family Rhodomelaceae[8] and of members of the green algal genus *Avrainvillea*[9]. Some of the first studies of marine algae documented the bromophenols present in red algae of the genus *Polysiphonia*[10].

The roles of the vidalols in *Vidalia obtusaloba* appear to be related to defense against diverse marine herbivores. Indeed, when coated on the palatable sea grass *Thalassia testudinum* and placed on coral reefs, vidalol A significantly reduced grazing by Caribbean herbivorous fishes[11]. In addition, and perhaps as expected of phenolic metabolites, vidalols A and B exhibited weak to moderate broad spectrum antibacterial activity against human pathogens and inhibited the marine pathogenic fungus *Lagenidium callinectes* in in vitro agar plate assays (50 µg/disk).

Vidalols A and B showed significant reduction in edema (58–82%) when applied topically to phorbol ester (PMA) – induced swelling of the mouse ear. In in vitro testing, vidalols A and B inhibited the arachidonic acid

metabolic pathway enzyme phospholipase A_2 (bee venom PLA_2) each showing 96% inactivation at 1.6 μg/ml[2]. Hence, vidalols A and B could provide interesting lead molecules for the design of inhibitors of this important enzyme.

Line many ortho-catechols, vidalols A and B slowly oxidize, in air, to yield red solutions and amorphous powders consisting of their corresponding ortho-quinones **4** and **5**. Because of the difficulty in maintaining vidalols A and B pure during bioassays, we are not sure if the catechols or the more reactive ortho-quinones exhibit the antibiotic and enzyme-inhibitory properties reported here. Indeed, in a similar case with the metabolites from the brown alga *Stypopodium zonale*[12], it was observed that air oxidation yielded an ortho-quinone which reacted as an electrophile binding sulfhydryl groups to selectively inhibit the enzyme tubulin[13].

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* Permanent address: Department of Chemistry, University of Iowa, Iowa City, Iowa, IA 52242.

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The phytotoxins of *Mycosphaerella fijiensis*, the causative agent of Black Sigatoka disease of bananas and plantains

A. A. Stierle, R. Upadhyay, J. Hershenhorn, G. A. Strobel* and G. Molina[a]

Department of Plant Pathology, Montana State University, Bozeman (Montana 59717, USA), and [a] Fundacion Hondurena de Investigacion Agricola APDO, Postal 2067, San Pedro Sula (Honduras)
Received 24 October 1990; accepted 16 January 1991

Abstract. Black Sigatoka is the most costly to control disease of bananas and plantains in the world. Currently, a worldwide search is underway either to find or to produce cultivars that are disease-resistant or -tolerant. Phytotoxins isolated from the pathogen might facilitate the discovery of such cultivars. Several aromatic compounds from liquid cultures of *Mycosphaerella fijiensis*, the causal agent of Black Sigatoka disease of bananas and plantains, have been isolated. The most abundant and phytotoxic of these compounds is 2,4,8-trihydroxytetralone, which induces necrotic lesions at 5 μg/5 μl in less than 12 h on sensitive cultivars of bananas. This compound exhibits host-selectivity that mimics that of the pathogen. Other phytotoxins isolated from this fungus, in lesser amounts, were juglone, the novel compound 2-carboxy-3-hydroxycinnamic acid, isoochracinic acid and 4-hydroxyscytalone. Some of the phytotoxins isolated are melanin shunt pathway metabolites, which makes this fungus unique among plant pathogens.
Key words. Epidemic; juglone; 2,4,8-trihydroxytetralone; melanin shunt pathway; plant pathogen; Sigatoka.

Bananas and plantains are the primary food source for millions of people in many areas of the world, including Central Africa, Southeast Asia, Central and South America, and the Caribbean. People in these regions are generally faced with high population growth and recurring food shortages, conditions that augment the importance of high yield, low cost crops like bananas and plantains[1]. They yield a sweet, nutritious fruit and produce a starch that can be used to prepare a variety of staple foods. Bananas and plantains also provide more than just complex carbohydrates. They also yield a diverse array of useful secondary products such as fibers, wrappers, confectioneries, vegetables, catsup, beer, wine and vinegar[1,2].