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RHIPOCEPHALIN AND RHIPOCEPHENAL; TOXIC FEEDING DETERRENTS FROM THE TROPICAL MARINE ALGA RHIPOCEPHALUS PHOENIX[1]

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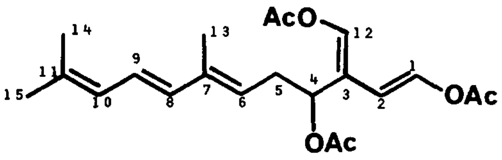
Green seaweeds of the families Caulerpaceae and Codiaceae abound in shallow tropical waters where herbivore feeding pressures are particularly intense.[2] Feeding preference studies and gut content analyses have shown that the predominant herbivorous fishes and urchins avoid consuming these plants despite their soft and potentially palatable forms.[3] While it is reasonable that marine plants parallel terrestrial plants in their evolved production of secondary substances to discourage predators, this mode of chemical adaptation has not been demonstrated in the marine environment. We wish to report here the isolation and structure elucidation of two new linear sesquiterpenoids, rhipocephalin (1) and rhipocephenal (2), from the Caribbean green alga *Rhipocephalus phoenix* (Ellis et Solander) Kuetzing[4] (Codiaceae). These compounds exhibit significant toxicity toward pomacentrid fishes,[5] and more importantly, 1 and 2 induce pronounced feeding avoidance behavior in the herbivorous Caribbean fish *Eupomacentrus leucostictus*.[6]

The freshly collected alga (Carrie Bow Cay, Belize, April, 1978) was immediately extracted ($CHCl_3$:MeOH, 1:1), and the condensed extract chromatographed on Si gel, in the field, to yield rhipocephalin (1, ≃ 0.1% wet weight), as a moderately unstable optically inactive oil (sodium D line). A molecular formula of $C_{21}H_{28}O_6$ was established for 1 by accurate mass measurement of the M^+-CH_2CO peak (obs. 334.1775; calc. 334.1780), in conjunction with the analysis of proton-decoupled and off-resonance-decoupled ^{13}C NMR spectra. Rhipocephalin showed IR absorptions for vinyl and alkyl ester carbonyl groups at 1770 and 1740 cm^{-1}, and UV absorptions (MeOH) at 257 (30,000), 266 (33,000), 277 (35,000) and 287 nm (27,000), characteristic of the conjugated triene and diacetoxybutadiene chromophores.[7] The ^{13}C NMR spectrum (20 MHz; $CDCl_3$) showed 1 was composed of six methyl groups at δ 12.7(q), 18.4(q), 2 × 20.6(q), 21.0(q), and 26.2(q), one methylene at 32.0(t), an acetate-bearing methine at 69.3(d), ten olefinic carbons at 109.5(d), 119.2(s), 124.2(d), 124.7(d), 125.6(d), 134.2 (d), 134.4(d), 135.2(s), 137.2(d), 137.2(s), and three ester carbonyl carbons at 167.3(s), 167.9(s), and 170.1(s). The eight degrees of unsaturation for 1, as indicated from the molecular formula, are accounted for by five double bonds and three ester carbonyls. Hence, rhipocephalin must be a linear sesquiterpenoid. Complete analysis of the 220 MHz ^1H NMR spectrum of 1, including rigorous spin-



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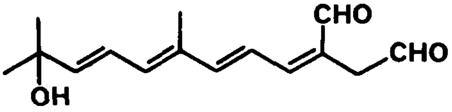

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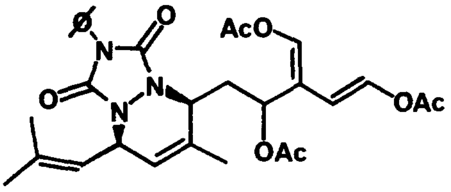

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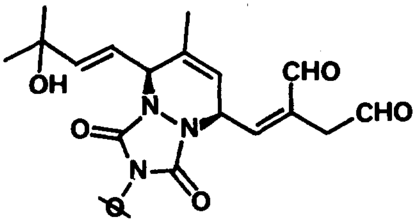

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decoupling experiments, led to the assignments of all protons in the molecule, as listed in Table 1. The geometry of the C-1 - C-2 olefin was assigned as E, based upon the comparable literature values of 12 Hz for <u>trans</u> protons in model compounds.[8] The C-3 - C-12 olefin stereochemistry was assigned as Z based upon the similarity of chemical shift of the C-12 proton with those in three 1,4-diacetoxybutadiene-containing compounds recently isolated from *Caulerpa* species.[7] The C-6 - C-7 double bond was assigned the E configuration based upon ^{13}C NMR values of δ 12.7 for the C-13 methyl

Table 1

220 MHz ^{1}H NMR SPECTRA OF 1 AND 2†

Rhipocephalin (1)

C#	δ $CDCl_3$	(C_6D_6)	m	J (Hz)
1	7.64	(7.98)	d	12
2	5.82	(5.80)	d	12
4	5.88	(6.26)	dd	7,7
5	2.71	(2.81)	ddd	14,7,7
5'	2.51	(2.58)	ddd	14,7,7
6	5.33	(5.49)	dd	7,7
8	6.09	(6.25)	d	15
9	6.36	(6.49)	dd	15,10
10	5.86	(5.93)	brd	10
12	7.22	(7.35)	s	
13	1.79	(1.79)	s	
14	1.79	(1.62)*	s	
15	1.79	(1.65)*	s	
OAc	2.16	(1.71)	s	
OAc	2.15	(1.58)	s	
OAc	2.06	(1.53)	s	

Rhipocephenal (2)

C#	δ $CDCl_3$	m	J (Hz)
1	9.60	t	2
2	3.53	d	2
4	7.19	d	12
5	6.48	dd	15,12
6	6.79	d	15
8	6.34	d	11
9	6.64	dd	15,11
10	6.05	d	15
12	9.51	s	
13	1.95	s	
14	1.38	s	
15	1.38	s	

* signals may be reversed

† allylic couplings of <1 Hz have been omitted

and δ 32.0 for the adjacent C-5 methylene, which illustrate the characteristic shielding of E olefins in spectrally well-known linear terpenoids.[9] Treatment of 1 with the dienophile 4-phenyl-1,2,4-triazoline-3,5-dione,[10] in $CHCl_3$ at room temp. for 1 min., gave high yields of the adduct 3 as a white solid. Analysis of the spectral characteristics for this compound illustrated that the addition to 1 involved the C-6 to C-9 diene system.[11] The facile nature of this reaction also supports the E assignments of the C-6 - C-7 and C-8 - C-9 olefins, since the cisoid diene conformation, necessary for the Diels-Alder reaction, would be more readily adopted when the terminal substituents are anti.

Silica gel column chromatography of the extract ($CHCl_3$:MeOH, 1:1) of frozen R. phoenix, stored for four days, failed to yield 1. However, smaller amounts (0.04% dry weight) of another sesquiterpenoid, rhipocephenal (2), were obtained after repeated chromatography on silica gel and finally bio-beads S-X8 (50% hexanes in CH_2Cl_2). High resolution mass measurement of the parent ion of 2 (obs. 248.1419, calc. 248.1412) established a molecular formula of $C_{15}H_{20}O_3$. Infrared absorptions (CCl_4) were observed for hydroxyl (3650, 3500 cm^{-1}), α,β,γ,δ-unsaturated aldehyde and normal aldehyde carbonyl functionalities (1660 and 1725 cm^{-1}), and for multiple unsaturation (1610, 1580 cm^{-1}). The UV spectrum of 2 in MeOH illustrated a maximum absorption at 350 nm(ε 45,000), which confirmed the presence of at least a tetraen-al chromophore (calc. for 2, 346 nm). The ^{13}C NMR spectrum (20 MHz, $CDCl_3$) was composed of the expected two aldehyde carbons at δ 197.5(d) and 193.0(d), eight olefinic carbons at 152.2(d), 148.9(d), 146.3(d), 137.7(d), 134.6(s), 131.4(s), 122.7(d), and 121.6(d), a quaternary oxygen-bearing carbon at δ 71.0(s), one methylene carbon at δ 39.4(t), and three methyl carbons, one at δ 12.6(q) and two overlapping at 29.8(q). The molecular formula for 2 illustrated six degrees of unsaturation. Since all six are accounted for by the two aldehyde carbonyls and four carbon double bonds, rhipocephenal must also be a linear sesquiterpenoid.

Complete analysis of the ^1H NMR spectrum of 2, involving extensive spin-decoupling studies, allowed the assignment of all protons and much of the stereochemistry of this molecule (Table 1). As one aldehyde proton was a triplet signal coupled to an adjacent methylene group, this functionality was readily assigned at C-1. The remaining nine non-methyl carbons of the linear skeleton must accommodate four double bonds. The unsaturation was indicated to begin at C-3 and extend to C-10 based upon several observations. Primarily, it was observed that the C-2 methylene group was allylically coupled to the lowest field olefin signal by ca. 1 Hz, and this band was, therefore, assigned at C-4. By virtue of its low field position, δ 7.19, the C-4 proton must be β-substituted to the aldehyde group. Hence, the tetraen-al chromophore was assigned involving carbons 12 and 3-10. The tertiary alcohol group, which failed to acetylate under mild conditions, was assigned at the terminus of the chain (C-11). This constellation was also characterized spectrally by two ^1H NMR methyl singlets at δ 1.38 and one ^{13}C NMR singlet at δ 71.0. The stereochemistries of the C-5 - C-6 and C-9 - C-10 olefins were clearly E (Table 1), and the C-3 - C-4 and C-7 - C-8 olefins were assigned as E based upon ^{13}C NMR features. The chemical shift of the C-13 methyl group, for example, was observed at δ 12.6, a position illustrating significant γ-shielding effects[9] from C-9. Using the same reasoning, the C-2 methylene was observed at δ 39.4, a position illustrating little shielding effects from C-5.

Treatment of 2 with the same dienophile[10] as in 1 also yielded an analogous addition product, 4. Interpretation of the spectral features of 4 showed that the cyclization had occurred at C-5 and C-8 of the tetraene system[12]. The facile nature of this reaction also supports the assignment of E stereochemistry to the C-7 - C-8 olefin, based upon aforementioned arguments.

Acknowledgments

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References and Notes

1. IMSWE (Inshore Marine Shallow Water Ecosystem) Project contribution No. 46.

2. J. C. Ogden and P. S. Lobel, *Environmental Biology of Fishes*, **3**(1), 49 (1978).

3. J. C. Ogden, *Aquatic Botany*, **2**, 103 (1976).

4. Voucher specimens of this alga, identified as *Rhipocephalus phoenix* by Dr. Jim Norris, have been deposited in the National Herbarium, Smithsonian Institution, Washington, D.C.

5. Toxicity was measured against the readily available marine fish *Pomacentrus coeruleus*, by treating replicate fish for 1 hour with concentrations of **1** and **2** between 2 and 10 μg/ml in seawater. If death did not occur within the first hour, the test fish were returned to clean seawater and monitored for chronic effects. Compound **1**, at a concentration of 2 μg/ml, caused loss of balance after 50 min. and death in 5 hours. Compound **2** showed no immediate effects at 10 μg/ml, but death occurred within 20 hours.

6. Feeding inhibition was measured by monitoring the feeding response (acceptance vs. rejection) of the marine fish *Eupomacentrus leucostictus*, an endemic Caribbean herbivore, toward food pellets (Longlife Shrimp-el-etts, Hartz Mountain Corp.), treated with various weight concentrations of **1** and **2**. The test compounds were applied to the pellets with diethyl ether and the solvent evaporated at 60° for 20 min. Food pellets treated with just the solvent were used as standards, and these were accepted by *E. leucostictus* 100% of the time. For both **1** and **2** the effective dose for 100% rejection (ED_{100}) was between 100-300 μg/g. It should be pointed out that this concentration range is *ca.* one-tenth the combined concentrations of **1** and **2** in the alga.

7. The *trans,trans*-1,4-diacetoxybutadiene functionality has recently been observed in closely related metabolites of the genus *Caulerpa*, see: A. J. Blackman and R. J. Wells, *Tetrahedron Lett.*, 2729 (1978), and V. Amico, G. Oriente, M. Piattelli, C. Tringali, E. Fattorusso, S. Magno, and L. Mayol, *Tetrahedron Lett.*, 3593 (1978).

8. M. E. Jung, *Chem. Commun.*, 956 (1974).

9. J. B. Stothers, *Carbon-13 NMR Spectroscopy*, Academic Press, New York, 1972, p. 433.

10. Produced from 4-phenylurazole, Aldrich Chemical Company #18, 895-6.

11. For **3**: IR ($CHCl_3$): 1720, 1750 cm^{-1}; UV (MeOH): 209, 253 nm; ^1H NMR ($CDCl_3$): δ 1.78(3H, s), 1.81 (3H, s), 1.90(3H, s), 1.96(3H, s), 2.09(3H, s), 2.15(3H, s), 2.53(2H, dd, J=7,7), 4.40(1H, bs) 5.08(2H, bm), 5.43(1H, bs), 5.80(1H, d, J=12), 6.17(1H, t, J=7), 7.21(1H, s), 7.4(5H, m), 7.61(1H, d, J=12); MS: m/e 551 (M^+).

12. For **4**: IR ($CHCl_3$): 3450, 1720, 1680 cm^{-1}; UV (MeOH): 210 nm: ^1H NMR ($CDCl_3$): δ 1.36(6H, s), 1.86(3H, s), 3.46(1H, d, J=17), 3.90(1H, d, J=17), 4.76(1H, bd, J=7), 5.01(1H, bd, J=9), 5.51(1H, bs), 5.65(1H, dd, J=16,7), 6.09(1H, d, J=16), 6.60(1H, d, J=9), 7.4(5H, m), 9.54(1H, bs), 9.75(1H, s); MS: m/e 423 (M^+).

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