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NOVEL PYRROLES FROM THE OREGON RED ALGA *GRACILARIOPSIS LEMANEIFORMIS*

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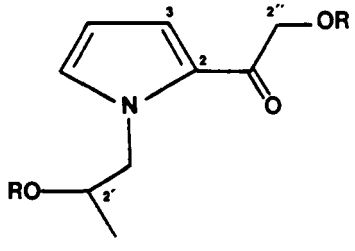
ABSTRACT.—Two new pyrrole natural products have been isolated from the temperate-water red alga, *Gracilariopsis lemaneiformis*, as diacetate derivatives. The structures of these *C*- and *N*-alkylated pyrroles were determined by spectroscopic methods (principally high field nmr and ms) to be *N*-(2-acetoxy)-propyl-2-(2-acetoxy)-acetopyrrole [**2**] and *N*-(2-acetoxy)-ethyl-2-(2-acetoxy)-acetopyrrole [**4**].

Over the past few years, our exploration of the natural product chemistry of cold- and temperate-water algae from the Pacific Northwest of the United States has revealed that many produce metabolites of polyunsaturated fatty acid origin (1–5). Other than tetrapyrrole pigments, pyrrole-containing natural products have been encountered only rarely in the marine environment, with distribution limited principally to sponges (6,7), bryozoans (8), cyanobacteria, and brown algae (6). Hence, we were encouraged to define the structures of two pyrrole natural products from the Oregon red alga, *Gracilariopsis lemaneiformis* (Bory) Dawson, Acleto et Foldvik (Gracilariaceae), despite their small yield from the alga. These were encountered during a study of the rich diversity of eicosanoid natural products obtainable from this seaweed, which is the subject of continuing investigation (4,5,9). Furthermore, pyrrole natural products **1** and **2**, while of fairly simple overall structure, posed some interesting problems in structure elucidation and are among only a few examples of *N*-alkylated pyrroles in nature (10–12).

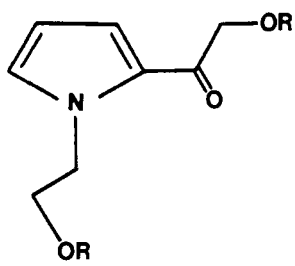
RESULTS AND DISCUSSION

A midsummer collection of the Oregon red alga *G. lemaneiformis* has been the subject of intensive investigation for its eicosanoid type natural products (4,5). In the process of isolating these unstable hydroxy acids, a series of fractions were derivatized with Ac_2O/pyridine and CH_2N_2. Following additional chromatography, a mid-polarity fraction contained a mixture of uv-active material which by nmr analysis were not of eicosanoid origin but rather contained low field bands indicative of a heteroaromatic structure. Following additional hplc separation, two acetate-containing pyrroles (**2**, 0.03%, and **4**, 0.02%) were isolated as very minor components of the lipid extract. While it is highly likely that these acetate groups originate from the derivatization procedure, the possibility of their natural occurrence cannot be unequivocally ruled out.

Derivative **2**, $[\alpha]^{23}D$ −47.2° (c = 0.29, MeOH), possessed multiple carbonyls



1 R=H
2 R=Ac



3 R=H
4 R=Ac

($\nu_{c=o}$ 1737.3, 1664.2 cm^{-1}), one of which was by uv analysis (λ max 286 nm) in conjugation with a pyrrole. Eims of derivative **2** gave an $[M]^+$ at *m/z* 267 which, in combination with ^{13}C- and ^1H-nmr data, provided a molecular formula of $C_{13}H_{17}NO_5$. Five of the six degrees of unsaturation inherent in this formula were accounted for by ^{13}C nmr as one conjugated carbonyl, two ester carbonyls, and four olefinic carbons. Hence, derivative **2** contained a single ring. The nature of this ring was evident from ^1H-nmr analysis, in which three aromatic protons were observed which were mutually coupled by small coupling constants typical of pyrrole rings. Confirmation of the proximate distribution of these three protons was shown by nOe difference spectroscopy (nOeds), in which positive enhancements were observed between each of these protons. Measurement of $^1J_{C\text{-}H}$ (Table 1) allowed unequivocal positioning of this spin system relative to the pyrrole nitrogen.

TABLE 1. ^1H and ^{13}C Nmr Data for Derivatives **2** and **4**.[a]

Position	Compound			
	2		**4**	
	^1H nmr	^{13}C nmr	^1H nmr	^{13}C nmr
2		126.98		127.00
3	6.98 (1H, dd, J = 4.3, 1.6 Hz)	119.30 (J_{CH} = 174.7 Hz)	7.01 (1H, dd, J = 4.3, 1.6 Hz)	119.43
4	6.17 (1H, dd, J = 4.1, 2.5 Hz)	108.84 (J_{CH} = 174.5 Hz)	6.19 (1H, dd, J = 4.1, 2.5 Hz)	109.10
5	6.90 (1H, d, J = 2.0 Hz)	131.60 (J_{CH} = 193.8 Hz)	6.92 (1H, d, J = 2.0 Hz)	131.66
$1'_a$	4.74 (1H, dd, J = 14.1, 3.0 Hz)	53.44 (J_{CH} = 143.6 Hz)	4.57 (2H, t, J = 5.3 Hz)	48.43
$1'_b$	4.14 (1H, dd, J = 14.1, 8.6 Hz)			
2′	5.19 (1H, m)	70.23 (J_{CH} = 152.3 Hz)	4.35 (2H, t, J = 5.3 Hz)	63.69
3′	1.25 (3H, d, J = 6.4 Hz)	17.36 (J_{CH} = 125.6 Hz)		
1″		182.77		182.67
2″a	5.22 (1H, d, J = 15.7 Hz)	65.23 (J_{CH} = 147.0 Hz)	5.15 (2H, s)	65.24
2″b	5.07 (1H, d, J = 15.7 Hz)			
acetates	2.22 (3H, s)	20.64	2.22 (3H, s)	20.61
	1.94 (3H, s)	21.00	2.01 (3H, s)	20.76
		170.52		170.62
		169.99		170.52

[a]All ^1H-nmr spectra were recorded at 300 MHz and ^{13}C nmr spectra at 75 MHz in $CDCl_3$ with internal TMS as a chemical shift reference.

Location of the conjugated carbonyl in **2** followed from consideration of uv, ir, and ^{13}C-nmr data in comparison with model compounds (13). Further, observation of nOe between the pyrrole C-3 proton and a deshielded methylene at C-2″ helped to define the nature of this two-carbon side chain as well as confirming its location at C-2 of the pyrrole. Furthermore, by ^1H- and ^{13}C-nmr chemical shift arguments (Table 1), an acetate functionality could be placed on this methylene (C-2″). By ^1H-^1H cosy another spin system was defined, which consisted of a propanol unit with an acetate functionality at

C-2′. This three-carbon unit was logically attached to the pyrrole nitrogen by virtue of a δ 53.44 shift at C-1′. Hence, the structure of derivative **2** was defined as *N*-(2-acetoxy)-propyl-2-(2-acetoxy)-acetopyrrole. Additional structural confirmation of derivative **2** came from an analysis of its eims fragmentation pattern (Figure 1).

The spectroscopic data (Table 1 and Experimental) for the diacetate derivative **4** were highly comparable to those obtained for derivative **2** and were related in molecular formula to **2** by subtraction of one methylene unit. The feature most notably absent in the ^{1}H-nmr spectrum for derivative **4** was the doublet methyl group (C-3′). This was replaced by an additional proton attached to C-2′ to form a 2H triplet at δ 4.35; hence derivative **4**, *N*-(2-acetoxy)-ethyl-2-(2-acetoxy)-acetopyrrole, was different from **2** in that it contained a two-carbon rather than three-carbon substituent on the pyrrole nitrogen. Interestingly, the change in the size of the substituent at this location in **4** results in reduced anisotropy of the C-2″ protons (two doublets in **2**, a sharp singlet in **4**).

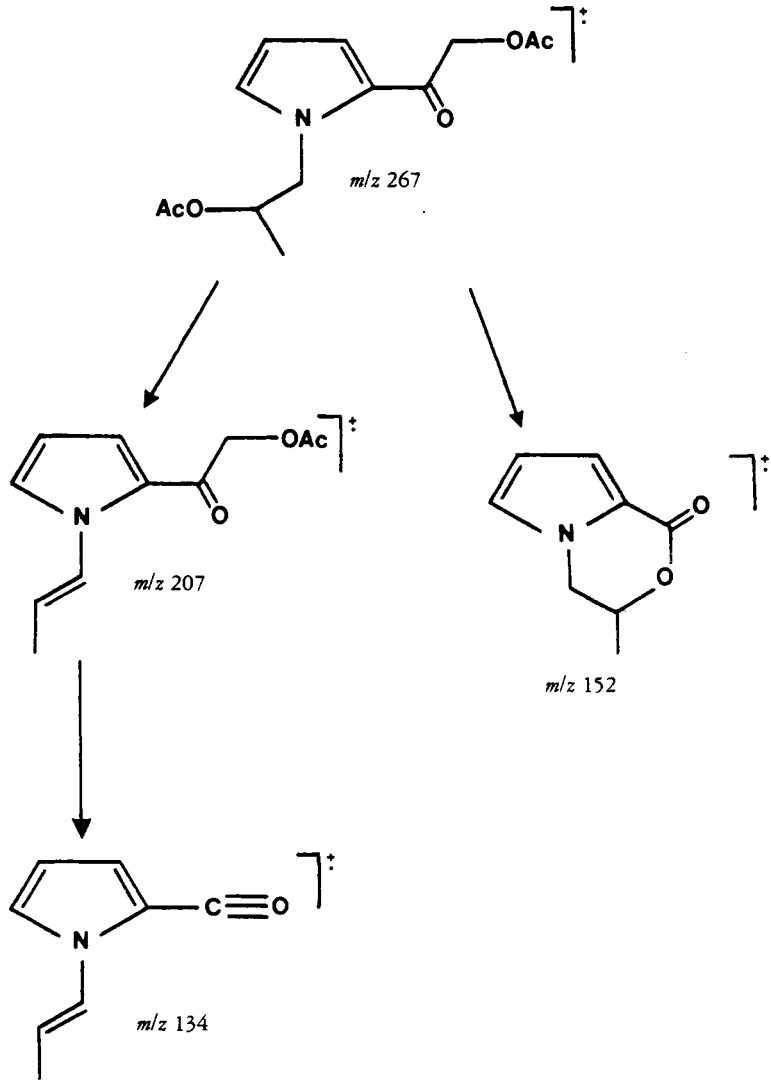


FIGURE 1. Proposed mass spectral fragmentation pattern of pyrrole derivative 2.

The eims fragmentation pattern for derivative **4** was highly analogous to that obtained for derivative **2** (see Experimental).

The linear and oxidized nature of the six-carbon chain (C-5 to C-2, C-1″, C-2″) in these metabolites is suggestive of an ultimate biosynthetic origin in sugar (hexose) metabolism, reminiscent of that of the recently described marine fungal metabolite leptosphaerin (14). Further, natural products **1** and **3** are members of a rare group of pyrroles in nature with *N*-alkyl substituents. The biosynthetic origin of these *N*-substituents likely involves products of intermediary metabolism. Unfortunately, the extremely small quantities isolated of these two metabolites have precluded their adequate biological evaluation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were recorded on a Beckman DB-GT UV vis spectrophotometer and ir on Nicolet 5 DXB FT 15 and Nicolet 510 spectrophotometers. Nmr spectra were recorded on a Bruker AC 300 NMR spectrometer, and all chemical shifts are reported relative to an internal TMS standard. Mass spectra were obtained on Finnigan 4023 mass spectrometer. Hplc was performed with Waters M-6000 and M-45 pumps, U6K injectors, and either an R401 differential refractometer or a Waters lambda-Max 480 lc spectrophotometer. Tlc used Merck aluminum-backed tlc sheets (Si gel 60 F 254). All solvents were distilled from glass before use.

COLLECTION, EXTRACTION, AND ISOLATION.—*G. lemaneiformis* was collected from exposed intertidal pools at Cape Perpetua on the Oregon coast on 1 July 1988, and a voucher specimen has been deposited in the herbarium of the University of California at Berkeley. The seaweed was immediately frozen with CO_2 (s) and stored frozen until workup. The defrosted alga (1.2 kg dry wt) was homogenized in $CHCl_3$-MeOH (2:1), steeped for 0.5 h, and filtered. The solvents were removed in vacuo to give 13 g of dark green oil. The crude organic extract (10 g) was acetylated overnight at room temperature with excess Ac_2O in pyridine (1:1). Pyridine and excess Ac_2O were removed in vacuo, and the acetylated material was fractionated by Si gel chromatography in the vacuum mode using a gradient of EtOAc in isooctane. The materials eluting with 30% EtOAc/isooctane were further purified on normal phase hplc (50 cm × 10 mm of RSil 10μ, 30% EtOAc/isooctane). Compound **2** was additionally chromatographed over reversed-phase hplc (25 cm × 10 mm 7μ LiChrosorb RP-18, 80% MeOH/H_2O), while **4** was subjected to final purification over reversed-phase hplc (25 cm × mm 7μ LiChrosorb RP-18, 70% MeOH/H_2O and then 50% MeOH/H_2O).

N-(2-ACETOXY)-PROPYL-2-(2-ACETOXY)-ACETOPYRROLE [**2**].—Derivative **2** was isolated as a colorless oil (2.9 mg, 0.03%): $[\alpha]^{23}D$ −47.2° (c = 0.29, MeOH); lreims (% rel. int.) $[M]^+$ *m/z* 267 (18), 207 (5), 152 (100), 134 (21), 108 (12), 94 (13); uv (MeOH) λ max 286 nm ($\epsilon = 5.3 \times 10^3$), 206 ($\epsilon = 1.8 \times 10^3$); ir (neat) ν max 2938.8, 1737.3, 1664.2, 1406.0, 1370.6, 1225.1, 1038.0, 946.7 cm^{-1}; ^1H and ^{13}C nmr see Table 1.

N-(2-ACETOXY)-ETHYL-2-(2-ACETOXY)-ACETOPYRROLE [**4**].—Derivative **4** was isolated as a colorless oil (2.0 mg, 0.02%): lreims (% rel. int.) $[M]^+$ *m/z* 253 (72), 193 (17), 180 (8), 138 (100), 120 (39), 94 (50); uv (MeOH) λ max 286 nm ($\epsilon = 9.1 \times 10^3$), 209 ($\epsilon = 2.5 \times 10^3$); ir (neat) ν max 2950, 1743.9, 1668.6, 1408.2, 1373.5, 1321.4, 1226.9, 1037.8, 943.3, 748.5 cm^{-1}; ^1H and ^{13}C nmr see Table 1.

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