Three New Sesquiterpenes from the Red Alga *Laurencia perforata*[1]

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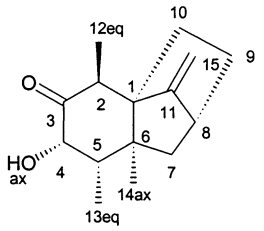
From the lipophilic extract of the red alga *Laurencia perforata* three new sesquiterpenes, (1*S**,2*S**,4*S**,5*S**,6*S**,8*R**)-4-hydroxy-2,5,6-trimethyl-11-methylenetricyclo[6.2.1.0^{1,6}]undecan-3-one (**1**, 4-hydroxy-1,8-epi-isotenerone), (3*R**,4*R**,4a*R**,9*R**)-3,9-dihydroxy-1,4,4a,7-tetramethyl-3,4,4a,5,8,9-hexahydro-2*H*-benzo[*a*]cyclohepten-2-one (9-hydroxy-3-epi-perforenone A, **2**), and 3-epi-perforenone A (**3**), were isolated. The structures of all isolates were determined from their spectroscopic data (NMR, MS, IR, UV). Unusual for a species of this algal genus was the apparent absence of halogenated compounds.

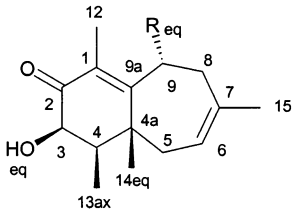
Algae belonging to the genus *Laurencia* (Rhodomelaceae) are some of the most prolific producers of secondary metabolites in the marine environment. To date, the scientific literature contains around 500 publications concerning the secondary metabolite chemistry of plants from this genus.[2] Secondary metabolites from these algae are predominantly sesquiterpenoid in origin and usually chlorinated and/or brominated.[2,3] There are, however, many reports concerning isolates that have other biosynthetic origins: diterpenes,[4] triterpenes,[5] acetogenins,[6] fatty acids,[7] and brominated indoles.[8] The ecological significance/functions of these secondary metabolites are less well investigated, but it is proposed that many behave as antifeedant principles. Antimicrobial[9] and cytotoxic[10] properties of a number of *Laurencia*-derived compounds may indicate many of these compounds to have multifunctional ecological roles. In the present study a sample of the red alga *Laurencia perforata* (Bory de Saint-Vincent) Montagne, collected from the waters around Magnetic Island (Great Barrier Reef, Australia), was investigated and found to contain three new sesquiterpenes (**1**−**3**).

The mass spectral data of **1** indicated it to have the molecular formula $C_{15}H_{22}O_2$ and thus five elements of unsaturation. Analysis of all of the spectroscopic data of **1** showed it to contain only two multiple bonds, a carbonyl group and a carbon−carbon double bond; the molecule was thus tricyclic. The second oxygen within **1** was clearly present as a secondary OH group on the basis of the IR absorption at 3495 cm^{-1} and the ^{1}H and ^{13}C NMR resonances at δ 3.82 (d, J = 3.0 Hz) and 79.1 d, respectively. After all protons had been assigned to their directly bonded carbon atoms, aided by an HMQC spectrum, it was possible to deduce the planar structure of **1** by interpretation of its ^{1}H−^{1}H COSY and HMBC spectral data. Thus, from the ^{1}H−^{1}H COSY spectra four spin systems could be discerned. Coupling between H_3-12 and H-2 gave the first, coupling between the exo-methylene protons gave the second, H_3-13 coupling with H-5, which further coupled with H-4, gave the third, and the fourth came from a continuous chain of coupling observed from H_2-7 through H_2-10. From the HMBC spectra of **1** long-range couplings seen between H_3-12 and C-1, C-2, and C-3 and between H-4 and C-3 and C-5 clearly showed the first two molecular fragments deduced above to be connected with each other via the carbonyl group C-3. Further HMBC correlations between the resonance for H_3-14 and those for C-1, C-5, C-6, and C-7, and between H_2-15 and C-1, C-7, C-8, C-9, C-10, and C-11, revealed the endo carbon (C-11) of the exo-methylene group to bond with C-1 and C-8, and C-6 to bond directly with C-1, C-5, C-7, and C-14, thus completing the planar structure of **1**. NOE difference measurements made with **1** produced diagnostic NOE interactions between H_3-14 and H-2, H-7a, H-10b, and H_3-13, which showed all of them, and the C-9−C-10 bridge, to be on the same side of the molecule, and H-2 and CH_3-14 to have axial orientations. Since H_3-14 had been established as being axial, H_3-13 must be equatorial, so as to enable the two groups to have the observed NOE interaction. This deduction meant H-5 must have an axial orientation, and since $J_{H-4,H-5}$ = 3.0 Hz, the 4-OH group must also be axially oriented. Thus, **1** is (1*S**,2*S**,4*S**,5*S**,6*S**,8*R**)-4-hydroxy-2,5,6-trimethyl-11-methylenetricyclo[6.2.1.0^{1,6}]undecan-3-one. Since **1** is the 4-hydroxy-1,8-epimer of isotenerone,[11] the trivial name 4-hydroxy-1,8-epi-isotenerone is proposed for the new molecule.



1

relative stereochemistry



2 R = OH
3 R = H

relative stereochemistry

9-Hydroxy-3-epi-perforenone A (**2**) analyzed for $C_{15}H_{22}O_3$ by CIMS and EIMS. Of the five degrees of unsaturation implied by the molecular formula of **2**, three were present

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Table 1. ^{1}H NMR Data (δ in ppm) for Compounds **1** (300 MHz, $CDCl_3$), **2** (400 MHz, $CDCl_3$), and **3** (300 MHz, $CDCl_3$)[a]

proton	1	2	3
2	3.29 (q, J = 6.8 Hz)		
3		4.21 (d, J = 5.1 Hz)	4.54 (d, J = 5.1 Hz)
4	3.82 (d, J = 3.0 Hz)	2.12 (dq, J = 5.1, 7.1 Hz)	2.15 (dq, J = 5.1, 7.1 Hz)
5	1.85 (dq, J = 3.0, 7.2 Hz)	2.17 (dd, J = 8.1, 16.3 Hz)	2.18 m
		2.32 (dd, J = 6.6, 16.3 Hz)	2.56 (brd, J = 2.0, 16.3 Hz)
6		5.32 (brdd, J = 6.6, 8.1 Hz)	5.44 m
7a	1.02 (d, J = 11.5 Hz)		
7b	1.53 (ddd, J = 2.8, 5.1, 11.5 Hz)		
8	2.25 (t, J = 5.1 Hz)	2.47 (dd, J = 11.2, 16.3 Hz)	1.98 m
		2.60 (dd, J = 5.6, 16.3 Hz)	2.26 m
9	1.29 m	5.09 (dd, J = 5.6, 11.2 Hz)	2.29 m
	1.68 m		2.59 (ddd, J = 3.1, 5.2, 12.6 Hz)
10a	1.70 (ddd, J = 4.7, 12.1, 12.1 Hz)		
10b	1.83 (ddd, J = 4.2, 4.7, 12.1 Hz)		
12	1.19 (d, J = 6.8 Hz)	2.07 s	1.84 s
13	0.99 (d, J = 7.2 Hz)	1.03 (d, J = 7.1 Hz)	0.86 (d, J = 7.1 Hz)
14	1.23 s	1.23 s	1.14 s
15	4.46 s, 4.68 s	1.70 s	1.76 s

[a] All assignments are based on extensive 1D and 2D NMR measurements including COSY, HMQC, and HMBC.

as double bonds: an α,β-unsaturated carbonyl group [253 nm (ϵ 3760), 1652 cm^{-1}, δ 200.5 s, 165.2 s, 128.6 s] and a carbon−carbon double bond; **2** is bicyclic. After the association of all protons with directly bonded carbons (HMQC spectrum) two protons remained unaccounted for. Clearly, these two protons were associated with the two remaining oxygen atoms in the form of two secondary hydroxyl functions (3435 cm^{-1}, δ 69.0 d, 73.4 d). From the ^{1}H−^{1}H COSY spectrum of **2**, three molecular fragments could be deduced: (1) C-3, C-4, and C-13; (2) C-5, C-6, and C-15; (3) C-8 and C-9. From the HMBC spectral data it was then possible to complete the planar structure of **2**. HMBC correlations observed between CH_3-12 and C-1, C-2, and C-9a, and between H-9 and C-1, C-4a, C-8, and C-9a, showed fragment 3 to bond via C-9 with C-9a, and CH_3-12 to bond with C-1, which further bonds with C-2 and C-9a. This new fragment was further enlarged to include fragment 1 on basis of the HMBC correlation observed between H-3 and C-2. From the HMBC correlations seen between CH_3-14 and C-4, C-4a, C-5, and C-9a, it was evident that CH_3-14 must bond with C-4a, which also bonds with the other three carbon atoms, and in so doing completes the six-membered ring within the molecule and leaves only the C-7, C-8 bond unaccounted for. The latter bond was confirmed from the HMBC correlation observed between CH_3-15 and C-8. NOE interactions between H_3-14 and H-9 and CH_3-13, and between H-3 and H_2-5, showed the 3-OH, CH_3-13, CH_3-14, and H-9 all to be on the same side of the molecule, as shown in **2**. From both the NOE data and the magnitude of the ^{1}H−^{1}H coupling constant between H-3 and H-4 (J = 5.1 Hz) it was evident that the 3-OH group must be equatorial, CH_3-13 axial, CH_3-14 equatorial, and H-9 axial. In a relative sense **2** is very similar to perforenone A,[12] the differences being the presence of an OH function at C-9 and the relative configuration at C-3 and/or C-4 ($J_{H-3,H-4}$ = 12 Hz,[12] indicates these two protons in perforenone A to be trans-diaxial).

Accurate mass measurement of **3** (3-epi-perforenone A) revealed it to have the molecular formula $C_{15}H_{22}O_2$. Comparison of all of the spectroscopic data of **3** with those of **2** showed the only differences between the two data sets to be the presence of resonances in both the ^{1}H and ^{13}C NMR spectra of **3** attributable to a methylene group at C-9 instead of the secondary hydroxyl function found in **2**. Stereochemically, **3** was judged to be identical, in a relative sense, to **2** on the basis of the similarity of the ^{1}H and ^{13}C NMR spectral data for both compounds at comparable stereocenters.

Table 2. ^{13}C NMR Data (δ in ppm) for Compounds **1** (75.5 MHz, $CDCl_3$), **2** (100 MHz, $CDCl_3$), and **3** (75.5 MHz, $CDCl_3$)[a]

carbon	1	2	3
1	56.3[b] s	128.6 s	125.6 s
2	37.6 d	200.5 s	200.1 s
3	213.0 s	73.4 d	72.7 d
4	79.1 d	42.0 d	46.2 d
4a		45.1 s	44.1 s
5	43.5 d	36.8 t	39.0 t
6	42.0 s	120.8 d	121.6 d
7	46.2 t	135.4 s	138.1 s
8	42.1 d	42.4 t	33.0 t
9	28.4 t	69.0 d	27.4 t
9a		165.2 s	165.9 s
10	27.0 t		
11	156.7 s		
12	8.8 q	12.5 q	10.9 q
13	11.1 q	10.2 q	9.1 q
14	17.7 q	24.3 q	24.2 q
15	100.8 t	26.1 q	26.6 q

[a] All assignments are based on extensive 1D and 2D NMR measurements including COSY and HMQC. [b] Implied multiplicity by DEPT (C = s, CH = d, CH_2 = t, CH_3 = q).

All compounds were tested for their potential antibacterial, antifungal, antialgal, antiparasitic, cytotoxic, and nematicidal activities, as well as for their abilities to inhibit the enzymes HIV-1-RT and tyrosine kinase,[13] and found to be devoid of any activity in these assays.

Experimental Section

General Experimental Procedures. The general experimental procedures were carried out as previously described.[14]

Collection and Isolation. Samples of *Laurencia perforata* were collected in the 1−4 m depth range from Magnetic Island, Great Barrier Reef, Australia, in August 1998. Collected material was stored at −20 °C until used. A voucher specimen, number MI189C, is stored at the Institute for Pharmaceutical Biology, University of Bonn, Nussallee 6, 53115 Bonn, Germany.

Plant tissue was freeze-dried (dry weight 74.0 g) and then exhaustively extracted with CH_2Cl_2 (4 L), followed by MeOH (4 L). Solvents were removed in vacuo, and the extracts filtered through a pad (10 mm thick) of silica gel. The CH_2Cl_2 extract (2.64 g, 3.6%) was separated by vacuum liquid chromatography (VLC, Si gel, gradient elution from petroleum ether−$(CH_3)_2$CO−MeOH−H_2O) to yield 24 fractions each of 60 mL. TLC and ^{1}H NMR examination of these fractions showed fractions 3 and 10 to be of further interest. HPLC spearation of fraction 3 using first a normal-phase Si column with petroleum ether−$(CH_3)_2$CO (9:1) as eluent, followed by separation with a

reversed-phase C18 column with MeOH−H_2O (4:1) as eluent, led to the isolation of **1** and **3**. HPLC separation of fraction 10 using a normal-phase Si column with CH_2Cl_2−$(CH_3)_2CO$ (4:1) as eluent yielded **2**.

(1*S,2*S**,4*S**,5*S**,6*S**,8*R**)-4-Hydroxy-2,5,6-trimethyl-11-methylenetricyclo[6.2.1.0^{1,6}]undecan-3-one (4-hydroxy-1,8-epi-isotenerone, 1):** oil (5.0 mg; 0.007%); $[\alpha]^{22}_D$ −60.0° (*c* 0.1, $CHCl_3$); IR ν_{max} 3495, 2945, 1707, 1460, 1195 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m*/*z* 234 (10) $[M]^+$, 216 (10), 165 (28), 147 (80), 133 (50), 119 (65), 109 (100), 91 (72), 79 (50); HREIMS *m*/*z* 234.1614 (calcd for $C_{15}H_{22}O_2$, 234.1614).

9-Hydroxy-3-epi-perforenone A (2): oil (8.8 mg; 0.012%); $[\alpha]^{22}_D$ −11.0° (*c* 0.2, $CHCl_3$); UV λ_{max} (EtOH) 253 nm (ϵ 3760); IR ν_{max} 3435, 2925, 1652, 1377 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; CIMS *m*/*z* 268 (100) $[M + NH_3]^+$, 251 (40) $[M + H]^+$; EIMS *m*/*z* 232 (2) $[M - H_2O]^+$, 206 (28), 182 (35), 153 (100), 135 (38), 125 (32); HREIMS *m*/*z* 232.1458 (calcd for $C_{15}H_{22}O_3$ − $[H_2O]$, 232.1458).

3-Epi-perforenone A (3): oil (4.0 mg; 0.05%); $[\alpha]^{22}_D$ −24.0° (*c* 0.1, $CHCl_3$); UV λ_{max} (EtOH) 253 nm (ϵ 8750); IR ν_{max} 3435, 2925, 1652, 1377 cm^{-1}; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively; EIMS *m*/*z* 234 (4) $[M]^+$, 206 (48), 173 (22), 164 (30), 137 (100), 123 (40); HREIMS *m*/*z* 234.1614 (calcd for $C_{15}H_{22}O_2$, 234.1614).

Biological Tests. Assays were carried out as previously described.[14]

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References and Notes

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