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TOXIC FEEDING DETERRENTS FROM THE TROPICAL MARINE ALGA *CAULERPA BIKINENSIS* (CHLOROPHYTA)

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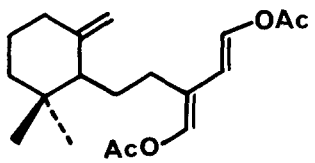
Abstract--Three new monocyclofarnesol-derived sesquiterpenoids with toxic and feeding deterrence properties have been isolated from the tropical green alga Caulerpa bikinensis from Palau Structure assignments for these new compounds are based upon spectral studies in comparison with related monocyclic sesquiterpenoids

Green algae of the family Caulerpaceae, represented by the single genus Caulerpa, are found world-wide, generally in shallow-water tropical and subtropical marine habitats. As these uncalcified algae are usually found in abundance and highly exposed in areas of significant herbivore populations, it seems likely that Caulerpa species possess chemical deterrents to reduce predation Chemical studies of various Caulerpa species have illustrated that some of these algae produce triterpenoids[1a], the nitrogen-containing compounds caulerpin[1b] and caulerpicin[1c] and the diterpenoid alcohol, caulerpol[1d] More recently, linear terpenoids possessing (or derived from) terminal E,E-1,4-diacetoxybutadiene functional groups have been isolated from several Caulerpa species[2] Unlike the former compounds, these latter substances, analogs of which are also isolated from related algae of the family Udoteaceae[3], appear to possess ichthyotoxic, cytotoxic, and feeding deterrent properties In this report, we wish to describe the structures of three new derivatives of this functional group class, isolated from the Pacific alga C. bikinensis W.R Taylor, collected in Palau, Western Caroline Islands The monocyclic terpenoids 1-3 are the first cyclic derivatives of this class isolated from green algae[4] Related substances such as onchidal (4), β-snyderol, and pallescensins 1-3, which possess similar carbon skeletons, have been isolated from the marine opisthobranch Onchidella binneyi[5a], the red alga Laurencia snyderae[5b] and the Mediterranean sponge Disidea pallescens[5c].

Standard silica gel column and high-performance chromatography of the $CHCl_3$/MeOH extract of C bikinensis[6] yielded 1-3 as 15, 5 and 1% of the extract, respectively. The major terpenoid, 1, was isolated as a viscous oil which showed $[\alpha]_D$ -3° (c=0 9, $CHCl_3$) A molecular formula of $C_{19}H_{28}O_4$ was established for 1 by a combination of high resolution mass spectrometry and ^{13}C NMR (Table 1). Absorption in the infrared spectrum of 1 at 1730-1760 and 1650 cm^{-1}, and in the ultraviolet spectrum at 245 nm (ε=26,000), in conjunction with the proton and ^{13}C NMR features which arise from C-1,



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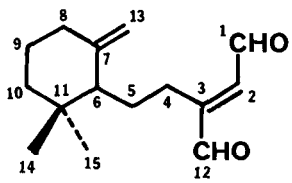

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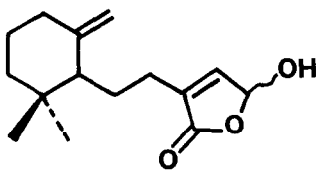

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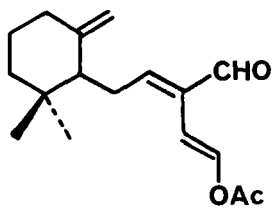

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C-2, C-3 and C-12, clearly indicated this metabolite to possess the well-known terminal E,E-1,4-diacetoxybutadiene functional group [2,3] The E configuration of the $\Delta^{3,12}$ olefin was further confirmed by the intensive nOe observed between the protons at C-2 and C-12, which is a feature already illustrated for the analogous configuration in caulerpenyne [2b] In addition to this latter functional group, the terpenoid 1 was recognized by its NMR features to possess one carbocyclic ring and an excyclic double bond (Table 1) Comparison of the ^{13}C NMR data for 1 with suitable models such as onchidal (4) and 10-debromosnyderol[5a], allowed the assignment of 1 to the well-known monocyclofarnesol class Particularly diagnostic were the ^{13}C NMR shifts for the gem-dimethyl groups, the quaternary carbon at C-11, and the methine carbon at C-6 which reflect the gamma-shielding effects inherent in this substituted cyclohexane ring system Further decoupling studies at 360 MHz allowed the majority of the protons in 1 to be confidently assigned

The dialdehyde 2, an oil, showed $[\alpha]_D$ +8.5° (c=0 8, $CHCl_3$), and analyzed for $C_{15}H_{22}O_2$ by ^{13}C NMR and HRMS (M^+ m/z obsd 234 1609, calc 234 1614). Strong infrared absorption at 1680 cm^{-1}, coupled with two ^{13}C NMR doublet bands at 191.6 and 195.3 ppm, and UV absorption at 227 nm (ε=3010), showed that 2 was an α,β-unsaturated dialdehyde The placement of the aldehyde functionalities at C-1 and C-12 followed mainly from analysis of proton NMR data The C-1 aldehyde proton was observed at δ 10 0 as a doublet (J = 7 5 Hz), coupled to the olefin proton at δ 5 46 (C-2). Since no further coupling was observed in the C-2 proton signal, and since both aldehyde

Table 1 ^{1}H and ^{13}C NMR Data for Metabolites 1-4 *

C#	1		2		3		4	
	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H [d]	^{13}C
1	δ7 38,d,J=12 3	[a]135 7d	10 0,d,J=7 5	[a]195 3d	6 06,bs	96 6d	8 26,d,J=14	141 2d
2	5 92,d,J=12 3	113 3d	5 46,d,J=7 5	140 6d	6 60,bs	142 6d	6 10,dd,J=14,1	105 8d
3	--	122 1s	--	151 1s	--	138 7s	--	135 2s
4	2 25m 2 05m	[b] 32 9t	2 60m 2 43m	[b] 32 4t	2 00m 2 30m	[a] 32 1t	6 41,t,J=7	156 6d
5	1 50m	[c] 24 9t	1 55m	[c] 26 2t	1 70m	[b] 29 6t	2 05,m	[a] 33 5t
6	1 70m	54 1d	1 74,dd,J=12,4	54 3d	2 30m	53 6d	2 57,m	53 5d
7	--	149 0s	--	148 5s	--	148 4s	--	148 1s
8	2 05m	[b]36 8t	2 05m	[b] 36 2t	2 00m	[a] 35 9t		[a] 37 4t
9	1 50m	[c] 24 2t	1 55m	[c] 23 7t	1 50m	[b] 23 9t	1 3-2 05m	[b] 24 8t
10	1 30m 1 45m	[c] 23 8t	1 2m 1 4m	[c] 23 3t	1 50-1 70m	[b] 23 6t		[b] 23 6t
11	--	35 0s	--	35 1s	--	34 8s	--	35 2s
12	7 15s	[a]134 2d	9 6s	[a]191 6d	--	171 6s	9 40,d,J=1	193 3d
13	4 84,d,J=2 3 4 64,d,J=1 0	109 1t	4 84,s 4 62,s	110 0t	4 76,s 4 54,s	109 7t	4 81,s 4 50,s	109 5t
14ax	0 81,s	25 7q	0 80,s	26 8q	0 80,s	26 3q	0 89,s	26 7q
15eq	0 92,s	28 5q	0 87,s	28 4q	0 86,s	28 2q	0 99,s	28 6q
OAc	2 15s	167 5s 20 5q	--	--	--	--	--	167 5s 20 6q
OAc	2 16s	167 1q 20 5q	--	--	--	--	--	--

* ^{1}H spectra recorded at 360 MHz and ^{13}C spectra recorded at 50 MHz, all in $CDCl_3$ solution Assignments of the ^{1}H NMR shifts for methylene protons (C4,C5,C8,C9,C10) are based upon decoupling results and predicted chemical shifts

a-c Indicates assignments may be reversed

d Recorded at 220 MHz in $CDCl_3$

functionalities were α,β-unsaturated, the second aldehyde was positioned at C-12 The E stereochemistry of the $\Delta^{2,3}$ olefin was readily determined by difference nOe techniques Irradiation of the C-12 aldehyde proton enhanced the C-2 olefin signal but did not affect the C-1 aldehyde, thus placing the aldehydes in a trans configuration. The cyclic structure of the remainder of the molecule was strongly indicated to be identical to 1 by its highly comparable ^{13}C NMR features. As in 1, proton decoupling experiments allowed the majority of the protons in 2 to be assigned.

The minor and most polar metabolite, 3, was isolated as a viscous oil which showed $[\alpha]_D$ -5.6° (c=0 8, $CHCl_3$), and analyzed for $C_{15}H_{22}O_3$ by HRMS (M^+ m/z obsd 250 1558, calc 250.1563) This metabolite was recognized as an α,β-unsaturated-γ-hydroxylactone by its overall spectral features which were almost identical to those of pallescensin-3.[5c] Infrared absorptions for hydroxyl (3400 cm^{-1}, br), and γ-lactone carbonyl (1760 cm^{-1}) were observed, along with only end absorption in the UV spectrum Features in the ^{13}C NMR spectrum assigned to this functional group included the lactone carbonyl (171 6 ppm, s), a trisubstituted olefin (142 6 ppm, d, 138.7 ppm, s) and a lactol carbon (96 6 ppm, d) Acetylation (AC_2O/py/RT) yielded the corresponding acetate, and resulted in a ^{1}H NMR shift of the C-1 lactol proton from δ 6 06 in 3 to δ 6.78 in the acetate. Another low-field band in the spectrum of 3 observed at δ 6.80 was assigned to the remaining olefin proton This was assigned to the β-position based upon its characteristic low-field position, and upon the small but unmeasurable coupling observed between the C-1 and C-2 protons. Here again, the remaining components of the structure of 3 were assigned in complete analogy with 1 and 2 by

comparison of their ^{1}H and ^{13}C NMR features.

Of the three sesquiterpenoids isolated from C bikinensis, it was mainly only the major metabolites 1 and 2 which showed biological activity The diacetate 1 and the dialdehyde 2 were found to be toxic to the Pacific damselfish Pomacentrus phillipinus at the 10 and 5 µg/ml levels Feeding deterrence effects were reliably produced from 1 and 2 when tested at 1000 ppm levels against similar herbivorous fishes The cytotoxicities of these compounds against the fertilized egg of the Pacific sea urchin Lytechinus pinctus were also measured. Again 1 and 2 showed ED_{50} values of 2 and 1 µg/ml. The activities noted for these metabolites reinforces their likely roles in nature as agents of chemical defense.

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