C-15 ACETOGENINS FROM THE MARINE ALGA *Chondria*

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ABSTRACT

(-) Z-Pinnatifidenyne, a novel C-15 acetogenin has been isolated along with the known compound (+)-3Z,6R,7R-obtusenyne and (+) (3Z)-laurenyne from the Australian red alga Chondria armata. The structures of the compounds were elucidated based on spectral data analysis including 2D NMR spectroscopic experiment.

Keywords: *Chondria armata, C-15 acetogenin, 2D NMR*

INTRODUCTION

The genus of *Laurenica* is the most intensively chemically investigated of all marine algae genera [1]. Algae belonging to this genus are extremely widespread and have well documented to produce diverse, unique secondary metabolites. The characteristic secondary metabolites from *Laurencia* can be classified into two based on their biogenetic pathway: terpenoid and non terpenoid. The first bulk group of metabolites isolated from these algae are sesquiterpenoid whereas the report of diterpenoids and triterpenoid is short. The vast majority of non terpenoid are halogenated C-15 acetogenin, which are based on a linear C-15 chain to cyclise ether rings of different sizes with terminal enyne function derived from the metabolism of fatty acid [2]. It is also interesting to note that several *Laurencia* metabolites halogenated C-15 acetogenin have also been found from *Ophistobranch* mollusks that feed upon this alga [3] and from the sponges of *Mycale rotalis* [4].

An abundant supply of C_{15} acetogenins has been found from marine red algae, genus *Laurencia*. In contrast, the occurrence of C_{15} acetogenin from the genus *Chondria*, algae of the same family as *Laurencia*, is very limited. In this paper we describe the isolation and structure elucidation of a novel halogenated C-15 acetogenin (-) Z-pinnatidinyne (**1**) along with the known compound 3(Z)-laurenyne (**2**) and (+)-*3Z,6R,7R*-obtusenyne (**3**) from *Chondria armata*, an alga of the same family of *Laurencia* (Rhodomelaceae, *Ceramiales)*.

EXPERIMENTAL SECTION

General

^{1}H NMR spectra were measured in $CDCl_3$ at 600 MHz and ^{13}C NMR spectra at 300 MHz on a Bruker NMR at the Australian Institute of Marine Science, Cape Ferguson. Optical rotation was observed on a Jasco P-1020 digital polarimeter. HPLC purifications were conducted by using reverse phase HPLC on a Hewlett-Packard C18 column (10 x 250 mm. Merck t.l.c. grade silica gel 5-40μ (type 60) was used for column chromatography. All solvents used were freshly distilled.

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Biological Material

Chondria armata algae were collected in Orpheus Island. Taxonomic identification was done by Prof. Rocky de Nys (school of marine biology and aquaculture, Jamescook University).

Extraction and Purification

Seventy point one gram (dry weight) was extracted three times with dichoromethane 250 mL. The solvent was removed on a rotary evaporator to afford a crude extract (0.629 g) which was rapidly chromatographic on silica gel under vacuum using a stepwise gradient from hexane to dichloromethane to ethyl acetate to methanol.

Fraction 1 was purified by reverse phase HPLC on a Hewlett-Packard C18 column (10 x 250 mm) eluted with acetonitrile 87% at a flow rate of 2 ml/min. The fraction with retention time 17.2 min was transferred to a separating funnel and extracted with dichloromethane. The water layer was removed and dichloromethane layer was evaporated under reduced pressure to afford 4.5 mg pinnatifidenyne (0.0064%)

Fraction 2 was a mixture of 3Z-laurenyne and obtusenyne. This mixture was separated by reverse phase HPLC on a Hewlett-Packard C18 column (10 x 250 mm) eluted with acetonitrile 75% at a flow rate of 2 ml/min. The fractions with retention time 24.3 min and 26.8 min were transferred to a separating funnel and extracted with dichloromethane. The water layer was removed and dichloromethane layer was further evaporated under reduced pressure to afford 3 Z laurenyne (13 mg, 0.019%) and *3Z,6R,7R*-obtusenyne (20 mg, 0.028%).

3Z-pinnatifidenyne (1). Colorless oil; $[\alpha]_D$ = -17.3 ($CDCl_3$, *c*. 0.03); ^1H and ^{13}C NMR data, Table 1.

3Z-laurenyne (2). Colorless oil; $[\alpha]_D$ = + 20 ($CHCl_3$, *c*. 0.01); $[\alpha]_D$ Lit = + 30.4 ($CHCl_3$, *c*. 0.63); ^1H and ^{13}C NMR data, Table 2.

(+)-3Z,6R,7R-obtusenyne (3). Colourless oil; $[\alpha]_D$ = + 20.7 ($CHCl_3$, c. 0.32); $[\alpha]_D$ Lit = + 10 ($CHCl_3$, *c*. 0.2) ^1H and ^{13}C NMR data, Table 3.

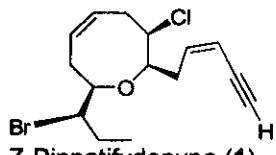
RESULT AND DISCUSSION

There is only one example of the isolation of a halogenated C_{15} acetogenin from the red alga of the genus *Chondria*. Chondriol (**4**) was known to be produced by *Chondria oppositclada*[5]. In contrast, *Laurencia* is unique in producing various C_{15} halogenated acetogenins. 3*E*- and 3*Z*- pinnatifidenyne (**1**) were previously isolated from *Laurencia pinnatifida*[6,7]. 3Z-Laurenyne has been reported from a new *Laurencia* species, L. *yonaguniensis*[8]. Moreover, the (3*E*)-isomer (**2**) had previously been found in *Laurencia obtusa*[9], and was also isolated from the Italian Sea hare *Aplysia punctata*[10]. Obtusenyne (**3**) was first reported from *Laurencia obtusa*[11].

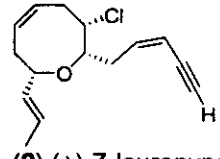
(-) (Z) Pinnatifidenyne (**1**) was purified through HPLC separations and was isolated as colorless oil. This compound was identified by comparison its ^1H and ^{13}C NMR spectra with previously reported data[6,7] as well as by 2D NMR techniques (HSQC, HMBC and COSY). The ^1H NMR spectrums exhibited signals for a terminal methyl group δ 1.08 (3H, t, J = 7.3 Hz, H_3-15), four olefenic protons, an acetylenic proton (δ 3.13, d, J = 2.3 Hz), two protons on oxygenated carbons, two protons on halogenated carbons and four methylenes (δ 1.79-2.96 integrating for 8H).

Two couple olefinic protons appeared at δ 5.69 (ddd J = 1.8, 6.5, 10.2, 12.1 Hz) and δ 5.91 (dddd J = 1.1, 7.2, 8.3, 10.3 Hz). The coupling constants ($J_{3,4}$ = 10.8 Hz) and the chemical shifts of an other two olefinic protons (δ 5.57 and 6.02) for H-3 and H-4 as well as the chemical shift value (δ_H 3.11) of the acetylenic proton indicated the geometry of the double bond at C-3 to be *Z*[6,12]. The bromomethine signals appeared at δ 3.98 (ddd J = 3.0, 5.4, 6.6 Hz) and the signal at δ 3.97 (ddd J = 2.6, 5.1, 10.4 Hz) was assigned to the chloromethine function.

^{13}C NMR spectra contained fifteen carbon signals attributed to one methyl, four methylenes, one quartenary carbon and nine methines carbons. The methine signals at 79.6 and 83.4 ppm established the ether linkage between carbons C-6 and C-12 indicating the presence of an oxocane ring in the molecule



Z-Pinnatifydenyne (**1**)



(**2**) (+) Z-laurenyne

Table1. ^1H and ^{13}C NMR assignment for (+) and (-) Z-pinnatifidenyne (**1**)

Position	(+)-Pinnatifidenyne reference			(-) Pinnatifidenyne sample			HMBC
	δ C	δH	Multiplicities	δ C	δH	Multiplicities	
1	82.6	3.13	d J = 2.0	82.49	3.13	d J = 2.3	C2,C3
2	80			79.94			
3	111.1	5.57	dd J = 2.0, 10.8	111	5.57	dd J = 4.7, 10.8	C5
4	140.6	6.03	dt J = 7.6, 10.8	140.86	6.02	dt J = 7.5, 10.8	
5	35	2.82	dt J = 7.6, 14.2	34.9	2.83	dt J = 7.9, 14.1	C3, C4, C7
5'		2.55	ddd J = 6.2, 7.6,14.2		2.51		C3, C4,C6
6	79.9	3.88	ddd J = 2.6, 6.2, 7.6	79.67	3.88	ddd J = 2.5, 5.7, 8.2	C5, C12
7	64.8	3.95	ddd J = 2.6, 4.2, 10.5	64.86	3.97	ddd J = 2.6, 5.1, 10.4	
8	34.4	2.51	ddd J = 1.1, 4.2, 12.1	34.38	2.52		C7, C9
8'		2.96	ddd J = 7.0, 10.5, 12.1		2.96	ddd J = 1.1, 10.1, 12.7	C10
9	128.8	5.69	ddd J = 1.1, 7.0, 10.2	128.76	5.69	ddd J = 1.8, 6.5, 10.2, 12.1	C8, C11
10	130.9	5.91	ddd J = 0.2, 7.6, 10.2	130.88	5.91	dddd J = 1.1, 7.2, 8.3, 10.3	C8, C11
11	30.5	2.37	ddd J = 3.0, 7.6, 14.0	30.09	2.37	ddd J = 1.15, 8.4, 13.9	C10, C12
11'		2.63	ddd J = 0.2, 10.2, 14.0		2.61		C10, C12
12	83.4	3.47	ddd J = 3.0, 4.1, 10.2	83.37	3.47		C6
13	61	3.98	ddd J = 3.4, 4.1, 11.5	61.03	3.98	ddd J = 3.0, 5.4, 6.6	C12
14	27.1	1.79	ddq J = 7.2, 11.6, 14.6	27.06	1.79	ddq J = 7.2, 10.1, 14.5	C13
14'		2.06	ddq J = 3.4, 7.2, 14.6		2.07	ddq J = 3.4, 7.2, 14.5	
15	12.8	1.08	t J = 7.2	12.8	1.08	t J = 7.3	C13, C14

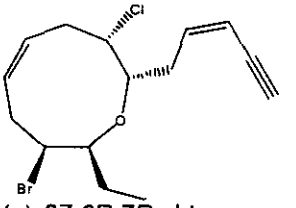
supported by long range correlations between H-6 (δ 3.88) and C-12 (δ 83.4) and in turn from H 12 (δ 3.47) and C-6 (δ 79.6). Four methines carbons at δ 140.86, 111.0, 128.75 and 130.88 correspond to two double bonds. Signals at δ 61 and 64.86 were assigned to bromomethine carbon and chloromethine carbon respectively. The assignments of these methines group are in agreement with literature data of C-15 acetogenins[6,7].

The presence of a terminal conjugated enyne group was suggested by long-range ^{1}H–^{13}C correlation between the acetylenic proton at δ 3.13 (δ_C 77.2, C-1) with quartenary carbon C-2 which appeared at δ_C 79.9 and C-3 (δ_C 111.0). All data are in accord with the ^{13}C NMR data of a C-15 acetogenin Z-pinnatifidenyne and other related compounds[6,7].

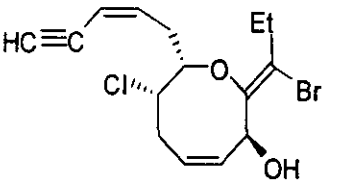
The NMR data of compound 1 (Table 1) were identical with those of Z-pinnatifidenyne isolated from *Laurencia pinnatifida*[6], while the sign of $[\alpha]_D$ value is opposite suggesting that (**1**) should be its enantiomer. It is note worthy that the (+) Z-pinnatifidenyne was isolated from red alga in the Atlantic Ocean (Tenerife island) while its enantiomer, (-) Z-pinnatifidenyne is produced by Australian red algae.

^{1}H and ^{13}C NMR data suggest that 3Z-laurenyne is a 7-membered cyclic ether with α- propenyl and α'-pentenynyl side chains. The presence of a 1-propenyl moiety was revealed by vinyl protons at δ 5.67 (1H, ddq, J = 15.3, 6.0, 1.5 Hz) and δ 5.55 (1H, ddq, J = 15.3, 6.0, 1.6 Hz) which were coupled to a methyl at δ 1.69 (3H, ddd, J = 6.3, 1.2, 1.0 Hz).The magnitude of coupling constant ($J_{13,14}$ = 15.2 Hz) for H-13 and H-14 indicated the geometry of the double bond at C-13 to be *E*. Moreover, the alkyne proton at δ 3.11 (1H, d, J = 2.2 Hz) and methine protons at δ 5.54 (1H, m), and δ 6.07 (1H, dddd, J = 10.9, 8.7, 6.3, 0.9 Hz) provided evidence for the presence of 2-penten-4-ynyl side chain. The coupling constants ($J_{3,4}$=10.9 Hz) for H-3 and H-4 as well as the chemical shift value (δ 3.11) of the acetylenic is only compatible with a *Z* configuration for the double bond which is conjugated with the triple bond[6,8,12]. Signals at δ_H 5.68 and δ_H 5.89 correspond to an additional double bond at C- 9 and C-10 respectively.

The nature of the heteroatom was readily recognised by correlating the assigned proton signals to carbons in the HSQC experiment. Hence, signals at δ_H 3.99 attach to the carbon with the chemical shift δ_C



(+)-*3Z,6R,7R*-obtusenyne (**3**)



Chondriol (**4**)

Table 2. ^{1}H and ^{13}C NMR assignment for (**2**) (+) Z-laurenyne

Position	Laurenyne reference			Laurenyne sample			HMBC
	δ C	δH	Multiplicities	δ C	δH	Multiplicities	
1	80.2	3.11	d, J = 2.2	82.04	3.11	d, J = 2.2	C3, C4
2	82.1			80			
3	110.2	5.54	m	110.1	5.54	m	C1, C5
4	141.8	6.07	ddd, J = 10.9, 6.5, 6.4, 1.0	141.54	6.07	dddd, J = 10.9,8.7,6.3,0.9	C2,C3,C5
5	35.3	2.73	dddd, J = 14.2, 8.8, 6.4, 1.5; Ha	35.15	2.72	dddd, J = 14.4, 9.2, 6.2,1.5	C3,C4, C6, C7
		2.55	dddd, J = 14.2, 8.5, 4.6, 1.0; Hb		2.54	dddd, J = 14.4, 8.7, 4.5, 0.9	C3, C4, C6
6	79.1	3.89	ddd, J = 8.8, 4.6, 2.4	78.92	3.89	ddd, J = 9.24, 4.4, 2.5	C4, C5, C12
7	65.3	3.99	ddd, J = 11.5, 4.9, 2.4	65.2	3.99	ddd, J = 11.4, 4.9, 2.5	C6, C8,
8	34.5	2.98	dddd, J = 12.7, 11.5, 10.0, 1.2; Ha	34.36	2.97	dddd, J = 12.5, 10.5, 10.2, 1.1	C6, C7, C9, C10
		2.53	m; Hb		2.52	m	C6,C7, C9, C10
9	128.6	5.68	dddd, J = 10.3, 10.0, 8.3, 1.5	128.39	5.68	m	C11
10	131.1	5.9	dddd, J = 10.3, 8.6, 7.3, 1.0	130.95	5.89	dddd, J = 10.4, 8.3, 7.1, 0.9	C7,C8, C11
11	34.9	2.47	m; Ha	34.74	2.47	dddd, J = 16.1, 9.1, 7.1, 1.1	C9, C10, C12
		2.15	ddd, J = 14.2, 8.6, 1.5; Hb		2.15	ddd, J = 14.2, 8.5, 1.6	C9, C10, C12
12	81.7	3.76	br dd, J = 8.8, 5.9	81.62	3.76	br dd, J = 8.9, 6.2	C6, C13, C14
13	132.1	5.55	ddq, J = 15.2, 5.9, 1.5	131.9	5.55	ddq, J = 15.3, 6.0, 1.6	C11, C14, C15
14	126.2	5.69	ddq, J = 15.2, 6.4,1.0	126.23	5.67	ddq, J = 15.4, 6.0, 1.5	C13, C15
15	17.8	1.69	ddq, J = 15.2, 1.0, 6.4	17.61	1.69	ddd, J = 6.3, 1.2, 1.0	C13, C14

65.2 ppm correspond to the chloromethine proton. Further more, the correlation between proton signals at δ_H 3.89 and the carbon at 78.92 and another correlation between signals at δ_H 3.76 and the carbon with chemical shift 81.62 indicated oxygenated carbons. Long range correlation between signals at δ_H 3.89 (H_6, ddd, J = 9.24, 4.4, 2.5 Hz) to C-12 and proton at δ_H 3.76 (H_{12}, br dd, J = 8.9, 6.2 Hz) to C-6 confirmed the ether bridge between C-6 and C-12.

The comparison of spectral data of compound **2** with those reported by Takahashi et al for 3Z-laurenyne indicated that **2** had the same structure and stereochemistry. The only exception observed was the different in chemical shift of carbon 1 and 2 (Table 2).

(+)-*3Z,6R,7R*-obtusenyne[3] was previously isolated from a South China Sea collection of Opisthobranch *Aplysia dactymela* Rang, 1828. It is interesting to report that this is the first isolation of (+)-*3Z,6R,7R*-obtusenyne from marine algae which indicate the mollusck may feed upon on this alga.

Anaysis of ^{1}H and ^{13}C NMR spectra confirmed the same enyne-containing side chain with Z-pinnatifidenyne and indicated a nine-membered cyclic ether skeleton. Comparison of the spectral data of compound **3** with those reported by Norte et al. (1991) for (3Z,12R,13R)-obtusenyne[7] and Manzo et al (2005) for *3Z,6R,7R*-obtusenyne[3] indicated that **3** had the same structure. However, both of the previous report did not show the chemical shift of C-2. Based on ^{13}C NMR spectra we confirmed that C-2 has the chemical shift 79.8 ppm supported by long-range ^{1}H–^{13}C correlation between alkyne proton H-1 (δ_H 3.16), methines proton H-3 (δ_H 5.56) and H-4 (δ_H 6.03) to carbon with the chemical shift 79.8 ppm.

The other differences were observed in ^{13}C NMR chemical shift of double bond carbons C-9 and C-10. Based on long-range ^{1}H–^{13}C correlation between the H-8 methylene proton at δ 2.46 to the double bond carbon (δ_C 129.9) and in turn from methine proton (δ_H 5.62, H-9) attach to this carbon to C-8 we suggest that C-9 was the carbon with chemical shift 129.9 ppm and C-10 was carbon with the chemical shift 128.4 confirmed by no correlation observed from methine proton (δ_H 5.61, H-10) to C-8. The two double bond carbons were exchangeable in both of the previous report [7,3].

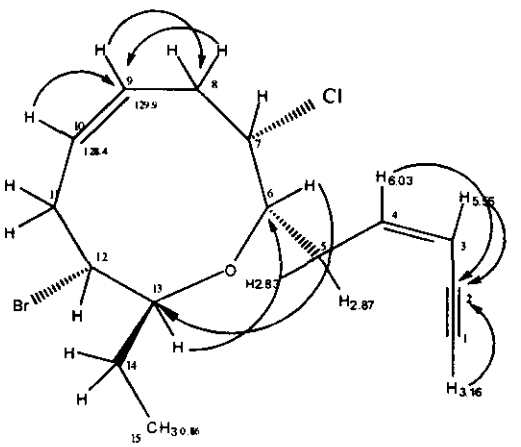


Figure 1. Selected HMBC correlation of (+)-*3Z,6R,7R*-obtusenyne

Table 3. ^{1}H and ^{13}C NMR assignment for (+)-*3Z,6R,7R*-obtusenyne (**3**)

Position	Obtusenyne reference			Obtusenyne sample			HMBC
	δ C	δH	Multiplicities	δ C	δH	Multiplicities	
1	82.8(CH)	3.15	d J = 2.4 Hz	82.2	3.16	d J = 2.8	C2, C3
2				79.8			
3	111.3 (CH)	5.56	d J = 10.6 Hz	111.2	5.56	d J =10.8	C1,C2, C4, C5
4	140.1 (C-H)	6.03	dt, J = 7.5, 10.6 Hz	140.1	6.03	dt J = 7.1, 10.8	C2,C3,C5,C6
5	34.7 (CH2)	2.84	m	34.5	2.83	m	C3, C6, C7
		2.84	m		2.87	m	C3, C4, C7
6	80.9 (CH)	3.58	m	80.9	3.58	m	C5, C8,C13
7	61.8 (CH)	4.06	m	62	4.06	ddd J = 3.3,5.5, 10.4	C8
8	33.5 (CH2)	2.44	m	33.4	2.46	m	C7, C9, C 10
		2.44	m		3.25	m	
9	128.6 (CH)	5.57	m	129.9	5.62	ddd J = 5.2, 10.5, 20.8	C8
10	130 (C-H)	5.6	m	128.4	5.61	ddd J = 5.3,10.6, 20.8	
11	34.1 (CH2)	2.59	m	34.2	2.58	m	C9, C10,C12
		2.59	m		3.4	m	
12	54.1 (CH)	4.26	m	54.5	4.25	m	C11
13	83.4 (CH)	3.22	m	83.5	3.23	m	
14	27.6 (CH2)	1.89	m	27.5	1.89	m	C6,C12,C13, C15
15	9.6 (CH3)	0.85	t J = 7.4 Hz,	9.68	0.86	t, J = 7.5	C13, C14

Another exception was Australian red alga has (+) $[\alpha]_D$ value while the Tenerife algae, *Laurencia pinnatifida*, has the opposite value. It is note worthy that this is the second examples of the metabolites produced by Atlantic ocean red algae having their enantiomer isolated from South Pacific ocean red algae.

CONCLUSION

From the Australian red alga Chondria armata, it has been isolated of (-) Z-Pinnatifidenyne, a novel C-15 acetogenin with the known compound (+)-3Z,6R,7R-obtusenyne and (+) (3Z)-laurenyne.

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