Anticancer Triterpene from the Marine Red Algae *Chondria armata*

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ABSTRACT

A triterpene, Intiricatetraol (1) was isolated from the marine red alga *Chondria armata* collected at Orpheus Island. The structures of the compounds 1 were established by extensive spectral data analysis including 2D NMR. Intiricatetraol (1) showed potent activity against P388 cell line with an IC₅₀ value of 41.8 nM.

Keywords: Triterpene, *Chondria armata*, cytotoxic, anticancer

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1. Introduction

Red algae of the genus *Laurencia* are known as prolific sources of halogenated terpenes. In contrast, the occurrence of terpenes from the genus *Chondria*, algae of the same family as *Laurencia*, is very limited. There are only several reports regarding the secondary metabolites from the marine red alga *Chondria armata* and only limited to diterpenes, triterpenes and C-15 acetogenins. Bromotriterpene polyethers were recently isolated from Indian sample *Chondria armata* (Ciavatta et al., 2001) and aplysiols C-E from Australian *C. armata* (Ola et al., 2010). The Australian collection of *C. armata* was also known to produce C15 acetogenin (Ola et al., 2008). Triterpene is one of the most interesting groups of marine natural products due to their chemical structure and pharmacological activity. Studies of the biological activity of these compounds have focused mainly on their potent cytotoxic effects (Suzuki et al., 1987; Suenaga et al., 1998; Norte et al., 1996; Fernandez et al., 2000).

We wish to report here the isolation and structure elucidation of another triterpene, intricatetraol, from the marine red alga of *Chondria armata*.

2. Materials and Procedures

2.1. Materials

The chemicals used in the experiments included methanol, CH₂Cl₂, ethyl acetate, distilled water, acetonitrile, etc. The equipments included HPLC, NMR, and ESI-MS.

2.2. Experimental Procedures

2.2.1. General procedure

Preparative high-performance liquid chromatography (HPLC) was performed using a GBC LC 1150 HPLC pump,

a Rheodyne 7725i manual injector with a 2.5 ml sample loop, and various C-18 columns (described under individual separation procedures). Samples were observed at multiple UV/Vis wavelengths simultaneously with a GBC LC 5100 Photodiode Array Detector (PDA) and WinChrom Chromatography Data System (Version 1.3.1) software. Flow rates ranged from 1-3 ml/min. Distilled water and LR grade Acetonitrile were filtered through a Millipore 0.45 µm HA (water) or FH (acetonitrile) membrane filter, and degassed by sparging with helium for 15 minutes. NMR spectra were measured at 300 MHz on a Varian Mercury spectrometer and at 600 MHz on a Bruker Avance spectrometer with cryoprobe. Optical rotations were observed on a Jasco P-1020 digital polarimeter. Electro spray ionisation mass spectrometry (ESI-MS) was performed in HPLC grade MeOH with a Bruker BioApex 47e Fourier Transform Ion Cyclotron Resonance Mass Spectrometer by Ms. Cherrie Motti at the Australian Institute of Marine Science, Townsville.

2.2.2. Plant material and extraction

Chondria armata was collected from Hazard Bay, Orpheus Island by Professor Rocky DeNys and frozen until extraction. The freeze dried alga (70.1 gram) was extracted three times with dichloromethane (CH₂Cl₂) 250 mL. The solvent was removed on a rotary evaporator to afford a crude extract (0.629 g) which was rapidly chromatographed on silica gel under vacuum eluted with 25 ml each of 25% CH₂Cl₂/Hexane, 50% CH₂Cl₂/Hexane, 100% CH₂Cl₂, 10% ethyl acetate (EtOAc) in CH₂Cl₂, 20% EtOAc/CH₂Cl₂, 50% EtOAc/CH₂Cl₂, 100% EtOAc, 10% methanol (MeOH) in EtOAc and 100% MeOH (fractions 1-9). Fractions 1 and 2

were purified through reverse phase HPLC to afford the acetogenins (Ola et al., 2008). The fraction eluted with 100% ethyl acetate was further purified through HPLC on a reverse phase column using 85% CH₃CN as the mobile phase to yield intricatetraol (3.1 mg) in pure form.

2.2.3. Cytotoxicity assays

Cytotoxicity was assessed *in vitro* against the P388D1 mouse lymphoma cell line using the sulphorhodamine (SRB) method (Houghton et al., 2007). The ability of the pure intricatetraol to inhibit the growth of the P388D1 mouse lymphoma cell line was assessed.

3. Results and Discussion

Chondria armata was collected in Orpheus Island and freeze dried. The dichloromethane extract (0.629 g) was subjected to vacuum liquid chromatography (VLC) on silica gel and fraction eluted with 100% ethyl acetate was further purified through HPLC on normal phase using 79 % CH₃CN as the mobile phase to yield *intricatetraol* (**1**) in pure form.

Intricatetraol (**1**), [α]_D²² +52.5 [c. 0.17 in CHCl₃], [α]_D²⁰ Lit +53.0 (Susuki et al., 1993)], was obtained as a colourless oil. The molecular formula of intricatetraol was determined to be C₃₀H₅₄Br₂Cl₂O₆ based on high resolutions ESIMS measurement. However, the ¹³C NMR data only exhibited 15 carbon signals so was indicative of a symmetrical structure. The ¹H NMR spectrum of intricatetraol contained signals for four methyl groups at δ 1.17, 1.26, 1.68 and 1.75, five methylenes, one halomethine proton at δ 4.09 (dd 10.9, 1.4 Hz), and two oxygenated protons at δ 3.58 (br d 10.1 Hz) and 3.85 (t 7.3 Hz). Both the ¹H NMR and ¹³C NMR data were more indicative of a sesquiterpene and thus indicated the presence of C₂ symmetry in intricatetraol.

Gradient selective TOCSY, HSQC and COSY experiments on the three methine protons allowed the three spin systems of the molecule to be identified. Thus, in the TOCSY experiment, irradiation of the methine proton at δ 4.09 induced signals from two methylene groups at δ 2.46

and 1.78 and at δ 1.75 and 1.48. The methine proton at δ 3.85 showed correlations with methylene proton signals at δ 1.98 and 1.91 and δ 2.17 and 1.53. The signal at δ 3.58 was coupled to a methylene group with signals at δ 1.82 and 1.78. These sequences were also confirmed by a COSY experiment.

HMBC data allowed the spin systems to be connected with the quaternary carbons bearing methyl groups. Hence, HMBC correlations from the methyl signals at δ 1.75 (H₃-25/30) and δ 1.68 (H₃-1/24) to the carbon resonances at 72.0 ppm (C-2/23) and 66.9 ppm (C-3/22) indicated these methyls are formed a gem-dimethyl group on a carbon bearing an electronegative heteroatom with an adjacent methine group that also carried an electronegative heteroatom. The *gem*- dimethyl group was also linked to the spin system of the methine proton at δ 4.09 on C-3/22 by strong correlation from the H-3/22 signal at δ 4.09 to the carbon at 72.0 ppm (C-2). The HMBC correlations from the methyl at δ 1.26 (H₃-26) to the quaternary carbon signals at 73.8 ppm (C-6/19), the methylene carbon at 37.1 ppm (C-5) and the methine carbon at 84.2 ppm (C-7) established the connections for the spin systems based on the methine protons signals at δ 4.09 (H-3/22) and δ 3.85 (H-7/18). The connection of the two spin systems was also confirmed by the correlation from H-7 to the methyl carbon signal at 24.2 ppm (C-26/29) and the quaternary carbon signal for C-6/19.

HMBC correlations from the methine proton at δ 3.58 (H-11/14) to the quaternary carbon resonating at 86.1 ppm (C-10/15) and the methylene carbon at 31.7 ppm (C-9/16) allowed the connection the above two spin systems. This was also supported by the correlation from the methyl signal at δ 1.17 (H₃-27/28) to the quaternary carbon resonating at 86.1 ppm (C-10/15), the carbon resonating at 31.7 ppm (C-9/16) and to the methine carbon signal at 77.6 ppm (C-11/14).

Figure 1. ¹H NMR spectrum for intricatetraol (**1**)

Fig. 1. Molecular structure of the *intricatetraol*

Table 1. NMR data for intricatetraol (1)

Position	Intricatetraol			Observed HMBC
	$\delta^{13}\text{C}$	$\delta^1\text{H}$	Multiplicities	
1(24)	27.5	1.68	S	C-2 (23), C-3 (22), C-25 (30)
2 (23)	72.0			
3 (22)	66.9	4.09	dd, 10.9, 1.4 Hz	C-1 (24), C-2 (23), C-5 (20)
4 (21)	28.9	1.78	M	C-2 (23), C-3 (22)
		2.46	M	C-6 (19)
5 (20)	37.1	1.48	M	
		1.75	M	
6 (19)	73.8			
7 (18)	84.2	3.85	t, 7.3 Hz	C-26 (29), C-8(17), C-6 (19)
8 (17)	26.6	1.98	M	C-7 (18), C-6 (19)
		1.91	M	
9 (16)	31.7	1.53	M	
		2.17	M	
10 (15)	86.1			
11 (14)	77.6	3.58	br d, 10.1 Hz	C-12 (13), C-9 (16), C-10 (15)
12 (13)	29.4	1.78	M	C-27 (28)
		1.82	M	
25 (30)	32.9	1.75	S	
26 (29)	24.2	1.26	S	C-5 (20), C-7 (18), C-6 (19)
27 (28)	23.9	1.17	S	C-9 (16), C-11 (14), C-10 (15)

According to the molecular formula, intricatetraol contained six oxygen atoms and required two degree of unsaturation. However, four oxygenated carbons (C-6/19 at 73.8 ppm, C-7/18 at 84.2 ppm, C-10/15 at 86.1 ppm and C-11 at 77.6 ppm) were observed in the ^{13}C NMR data for half of the molecule, which suggested an ether bridged linkage between C-7 and C-11. The presence of this ring was established from the mutual NOESY correlations between the methyl signal at δ 1.17 (H_3 -27/28) and the methine proton at δ 3.85 (H -7/18) despite the absence of an HMBC correlation.

The NMR data of this metabolite was very similar to those reported for intricatetraol isolated from *Laurencia intricata* (Suzuki et al., 1993). However, some differences were observed in the H and ^{13}C NMR data. In the H NMR spectrum, the differences were observed for signals from the methylene protons H_2 -5/20 at δ 1.75 and 1.48, the H_2 -12/13 protons at δ 1.82 and 1.78 and H_2 -8/17 at δ 1.91 and 1.98. These protons were previously reported with a single proton resonance frequency in each case i.e they were reported to

resonate at δ 1.50 (H_2 -5/20), δ 1.75 (H_2 -12/13) and at δ 1.90 (H_2 -8/17). In ^{13}C NMR data, the difference was observed for C-3 (66.9 ppm) which was previously reported at 67.5 ppm. This is likely to be concentration effect. The similarity of the NMR data and the value of the specific optical rotation suggested the metabolite is in fact intricatetraol. The relative stereochemistries of two oxalane rings in intricatetraol isolated from *Laurencia intricata* were proposed by the authors of the first report (Zusuki et al., 1993) and the absolute stereochemistry of intricatetraol was determined by chemical synthesis (Yoshiki et al., 2007).

As the crude extract showed cytotoxic activity, further pharmacological study focused on cytotoxic effect was undertaken. Interestingly pure intricatetraol (1) showed potent activity against P388 cell line with an IC_{50} 41.8 nM.

4. Conclusion

A triterpene, Intricatetraol (1) was isolated from the marine red alga *Chondria armata* collected at Orpheus Island. This triterpene showed potent cytotoxic activity against P388 cell line with an IC_{50} value of 41.8 nM.

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