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Chemical investigation of seven Australasian *Cystophora* species: New chemistry and taxonomic insights

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ABSTRACT

Chemical investigation of seven species from the brown algal genus *Cystophora* collected throughout Australia and New Zealand resulted in the isolation of two new compounds, 9,10-farnesylacetone epoxide **(1)** from *Cystophora moniliformis* and 1′-(2′,4′,6′-trihydroxyphenyl)-13-hydroxyoctadeca-6*Z*,9*Z*,11*E*,15*Z*-tetraen-1-one **(12)** from *Cystophora scalaris*, and 16 others that have been previously reported. Structures were elucidated using spectrometric methods, particularly 1D and 2D NMR spectroscopy. Taxonomic implications of the chemical findings are discussed including the possibility that **1** may be an intraspecific marker for the Western Australian population of *C. moniliformis*, and that **12** may be diagnostic for populations of *C. scalaris* from New Zealand waters.

1. Introduction

It is well known that the morphology of marine macroalgae can be quite plastic, and may be dependent upon such factors as light intensity, wave action, and position in the water column. This can result in difficulties in identification when using only morphological based taxonomy; classic examples of this phenomenon have been reported for the genera *Fucus* (Kalvas and Kautsky, 1998; Sideman and Mathieson, 1985) and *Dictyota* (Teixeira et al., 1990; Vallim et al., 2005). The morphological based taxonomy of the brown algal genus *Cystophora* has been considered to be stable for some time (Womersley, 1964) but some concerns about affinities within the genus have been raised, particularly the presence of *Cystophora fibrosa* in the genus (Womersley, 1987) and the relatedness of *Cystophora retorta* and *Cystophora siliquosa* (Kraft, Pers. comm.). Investigations into the chemistry of a number of *Cystophora* species have given some credence to these concerns. In particular, the isolation of a series of tetracyclic meroditerpenoids from the sole South African member of the genus, *C. fibrosa*, has been used to argue for the reassignment of this species to the closely related genus *Cystoseira* (Laird and van Altena, 2006).

Noting that chemotaxonomic approaches had previously been successful in the Phaeophyceae (Amico, 1995; Valls and Piovetti, 1995; Vallim et al., 2005), it was felt that a similar approach could be applied to understanding the taxonomic affinities within the *Cystophora* (family Sargassaceae (formerly Cystoseiraceae, now subsumed into the Sargassaceae): Fucales: Phaeophyceae). A review of *Cystophora* chemistry suggested that a chemotaxonomic approach could be used to

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differentiate species within the genus as a result of the production of distinct structural classes in different species. For example, *Cystophora moniliformis* almost exclusively produces farnesyl and geranylgeranyl isoprenoid derivatives (Kazlauskas et al., 1978; Ravi et al., 1982; Reddy and Urban, 2008; van Altena, 1988); *Cystophora brownii* has been shown to contain unique meroditerpenoids (Bian and van Altena, 1998) and *Cystophora harveyi* contains a unique selection of toluquinols (Capon et al., 1981; Laird et al., 2007). What was also obvious from this literature was that the chemistry of only a relatively few species had been described. The results presented below are from an ongoing effort to gain a more complete understanding of the chemistry of *Cystophora* and how that information can be used to distinguish individual species and establish an overall biochemical picture from which an evolutionary lineage in the genus may be inferred.

Specimens for this study were collected from various locations ranging from the southwest of Western Australia to New Zealand. This study reports the first chemical investigation of *Cystophora intermedia* and *C. retorta*.

2. Materials and methods

2.1. General

2.1.1. Collection

All species were collected by SCUBA diving or snorkelling at depths ranging from inter-tidal rock pools to 5 m from the areas listed below:

C. moniliformis, *Cystophora harveyii*: Sarge Bay, Cape Leeuwin, Western Australia (WA) (33° 22′ S, 115° 08′ E) in November 1998.

C. retorta: Cowaramup Bay, Western Australia (33° 51′ S, 114° 59′ E) in November 1998.

Cystophora scalaris: northern shore of Cook Strait, east of Wellington, New Zealand (coastline from 41° 21′ S, 174° 45′ E to 41° 20′ S, 174° 47′ S) in December 2000.

C. ystophorasubfarcinata: north-eastern West Island (The Amphitheatre), South Australia (SA) (35° 36′ S, 138° 35′ E) in May 1989, recollected Victor Harbor, South Australia (35° 35′ S, 138° 36′ E) in August, 1991.

C. siliquosa: large tidal pools on a wave cut platform at Sorrento Back Beach, Victoria (38° 18′ S, 144° 42′ E) in January 2001.

C. intermedia: intertidally exposed rock platform, north-eastern West Island (The Amphitheatre), South Australia (35° 36′ S, 138° 35′ E) in August 1989.

Cystophora torulosa: northern shore of Cook Strait, east of Wellington, New Zealand (coastline from 41° 21′ S, 174° 45′ E to 41° 20′ S, 174° 47′ S) in December 2000.

Algal material was kept on ice until return to the laboratory where it was frozen at −20 °C until required for extraction. Field identifications were confirmed by local experts: Gerald Kraft (University of Melbourne; Victorian collection), Allan Millar (Royal Botanic Gardens, Sydney; NSW and SA species), Wendy Nelson (Te Papa tongawera, Wellington; New Zealand collection), and John Huisman (Murdoch University; WA species). Voucher specimens of *C. siliquosa* (sample identification: SB070201-4), *C. intermedia* (WI890818001), *C. retorta* (MURUCW981104002), *C. torulosa* (WNZ281100-002), and *C. scalaris* (WNZ281100-006) are maintained within the Marine Natural Products Group, Discipline of Chemistry, The University of Newcastle and the Murdoch University Herbarium, Western Australia for *C. harveyi* (MURUAA981104004). Visual identification of *C. moniliformis* is completely unambiguous (Womersley, 1987; van Altena, 1988).

2.1.2. Spectroscopy

All solvents were spectroscopic grade unless stated otherwise. IR spectra were recorded as chloroform casts on NaCl plates using a Perkin Elmer Paragon 1000 FT-IR spectrometer between 4000 and 400 cm^{-1} with dry AR $CHCl_3$ as solvent. UV spectra were recorded as ethanolic or methanolic solutions on a Hitachi U-2000 UV–visible spectrophotometer. Optical rotations were recorded in $CHCl_3$ on a Perkin Elmer 241 Polarimeter. NMR data was collected with a Bruker Avance 300 DPX NMR spectrometer and utilised solvent (CDCl3) signals as an internal calibration standard (residual ^1H δ_H 7.24 *s* and δ_C 77.0 *t*). Field strength for ^1H NMR was 300.13 MHz and 75.5 MHz for ^{13}C NMR spectra. HMBC and HMQC experiments utilised the standard Bruker gradient pulse programs. HREIMS was performed by Dr. Noel Davies and Mr. Marshall Hughes of the Central Science Laboratories, University of Tasmania, Hobart, Australia, on a Kratos Concept IQ mass spectrometer with 70 eV ionisation and 5.3 kV accelerating voltage. Low-resolution MS were obtained using a Shimadzu GC-17A gas chromatograph coupled to a Shimadzu QP5050A quadrapole mass spectrometer.

2.1.3. Chromatography

All bulk solvents were distilled from glass prior to use and separations utilising HPLC were performed with HPLC grade solvents that were filtered (0.45 μm) and degassed prior to use. Light petrol is the 60–80° boiling fraction of supplied bulk 'mixed hexanes'. TLC was conducted on Merck 0.2 mm silica gel 60 F254 aluminium backed TLC plates. Gel permeation chromatography was conducted with Sephadex LH-20 (Pharmacia) at atmospheric pressure using a column 35 mm in diameter and approximately 700 mm in length. Solvent flow was approximately 1.5 mL min^{-1} and fractions were collected every 7 min. 'Speedy' column chromatography utilised Merck silica gel H (20–25 μm) and centrifugal chromatography employed a Chromatotron (Harrison Research) with plates constructed from Merck silica gel silica gel 60 F254 containing gypsum. Preparative TLC was performed using Merck glass PLC plates (20 × 20 cm, 2 mm silica gel 60 F254). All semi-preparative HPLC was normal phase and accomplished utilising a Waters 600 Controller coupled to a Waters PDA detector