1. Dynamin-I Inhibitors from Sessile Marine Invertebrates; 2. Chemotaxonomy of *Cystophora* spp.

Doctor of Philosophy (Chemistry)

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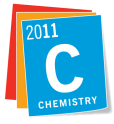

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Ian P. Holland

July, 2011

~ Somewhere, something increadible is waiting to be known ~

Dr. Carl E. Sagan, 1934–1996

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To my parents, Bob and Coleen

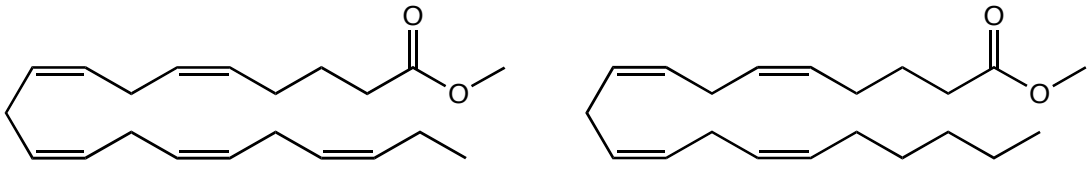
Abbreviations and Symbols

1D	One-dimensional
2D	Two-dimensional
Å	Angstroms
APCI	Atmospheric pressure chemical ionisation
ax	Axial
$[\alpha]_D$	Specific optical rotation
br	broad (in NMR)
BuOH	Butanol
^{13}C NMR	Carbon nuclear magnetic resonance (spectroscopy)
C_5D_5N	Deuterated pyridine
C_6D_6	Deuterated benzene
$CDCl_3$	Deuterated chloroform
$CHCl_3$	Chloroform
CH_2Cl_2	Dichloromethane
CMT	Charcot-Marie-Tooth
CNM	Centronuclear myopahy
COSY	Correlation spectroscopy
δ	Chemical shift in ppm
DEPT	Distortionless enhancement by polarization transfer
DHA	Docosahexanenoic acid
ϕ	Dihedral angle
EI	Electron impact
EPA	Eicosapentaenoic acid
eq	Equatorial
EtOAc	Ethyl acetate
ESI	Electrospray ionosation
FT	Fourier transform
GC	Gas chromatography
GI	Growth Inhibition

^{1}H NMR	Proton nuclear magnetic resonance (spectroscopy)
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum coherence
HPLC	High performance liquid chromatography
Hz	Hertz
i-octane	2,2,4-trimethylpentane
IC_{50}	Half Maximal Inhibitory Concentration
IR	Infrared
J	Coupling constant
λ_{max}	Absorbance maximum
LC	Liquid chromatography
MeOD	Deuterated methanol
MeOH	Methanol
MiTMAB	Myristyltrimethylammonium bromide
MS	Mass spectrometry
mult	Multiplicity
m/z	Mass to charge ratio
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser enhancement
NOESY	Nuclear Overhauser effect spectroscopy
OcTMAB	Octadecyltrimehtylammonium bromide
Petrol	Light Petroleum (60°C – 80°C fraction)
ppm	Parts per million
PUFA	Polyunsaturated fatty acid
t_R	Retention time
SAR	Structure-activity relationship
Sp.	Species
TLC	Thin-layer chromatography
UV	Ultraviolet

Abstract

A colourimetric GTPase assay was utilised to screen a marine natural products library of extracts with the goal of identifying novel dynamin-I inhibitors. Bioassay-guided fractionation of an active marine sponge (unknown Australian species) fraction led to the isolation of the methyl esters of eicosapentaenoic acid (EPA) (**15**) and Arachidonic acid (**16**). These compounds are structurally similar to the known dynamin-I inhibitors MiTMAB (**1**) and OcTMAB (**2**), however, **15** and **16** were inactive in the dynamin-I GTPase bioassay.



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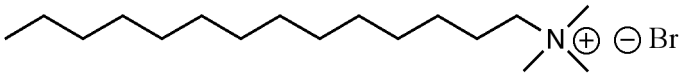

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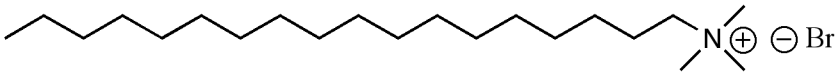

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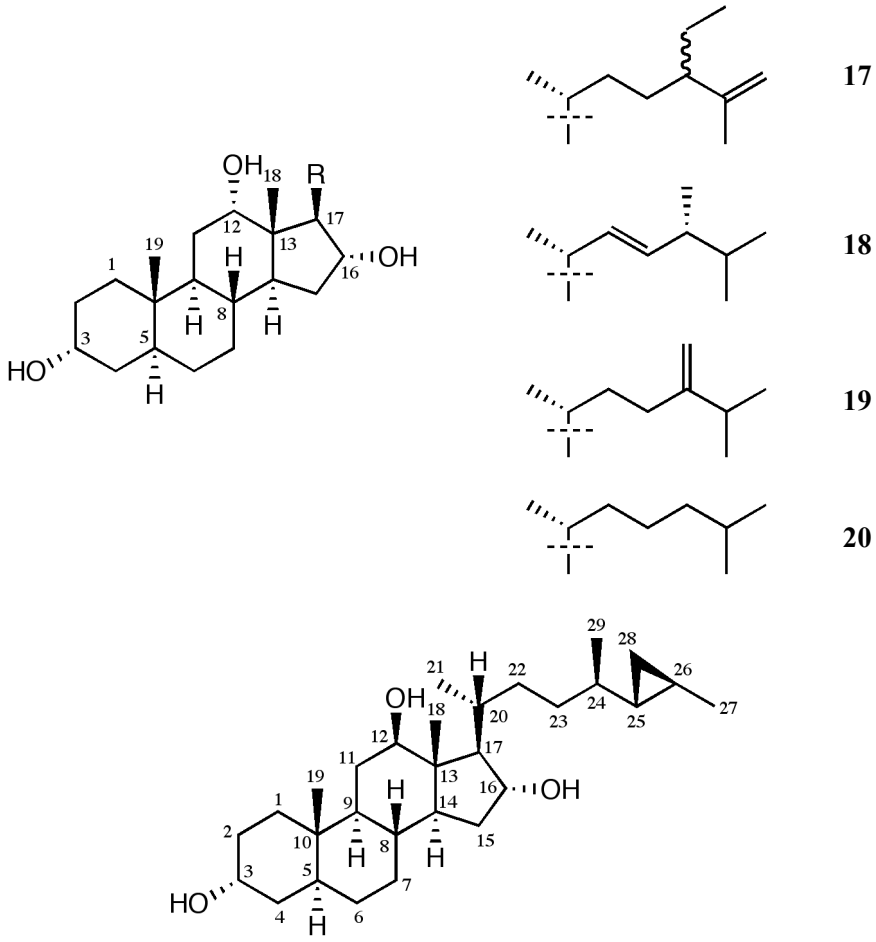
An extract of another Australian marine sponge *Psammocelmma* sp. was found to possess dynamin-I inhibitory activity. Bioassay-guided fractionation led to the isolation of four new trihydroxysterols (**17**–**20**) related to aragusterol G (**22**). While **17** was inactive in the dynamin-I bioassay, bioassays did reveal that compounds **17**–**20** inhibited the growth of colorectal, breast, ovarian and prostate cancer cell lines (GI_{50} 5–27 μM). The additional insight that these new compounds provide to previous SAR studies is also discussed.



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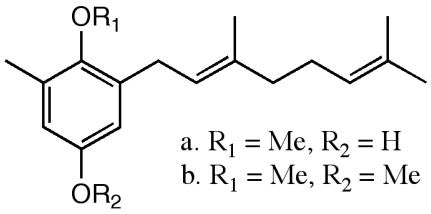
In addition, the chemistry of the brown algae *Cystophora torulosa* and *C. xiphocarpa* were investigated. It is well known that *C. torulosa* produces a range of secondary metabolites including resorcinols, tocotrienols, polyunsaturated alkene chains and phloroglucinols. Considering these are fairly 'standard' *Cystophora* compounds that have also been isolated from apparently closely related *Cystophora* species, the isolation of the previously discovered meromonoterpenes **51**a, **52** and **53** was unusual. Since these meroterpenoids potentially could be used as geographic marker compounds for New Zealand populations of *C. torulosa* and suggest an unexpectedly close relationship with *C. harvei* it was judged necessary to confirm the isolation of the meromonoterpenoids from *C. torulosa*. *Cystophora torulosa* was reinvestigated with the aim of re-isolating **51**a, **52** and **53**. This investigation yielded many of the known *C. torulosa* compounds including polyenes (**40** and **41**), an isoprenyl chroman (**48**), a resorcinol (**42**) and fucosterol (**92**) but not the meromonoterpenes in question. It is apparent

from comparison of the TLC profile of the crude *C. torulosa* extract with the isolated compounds that this investigation has not yet been exhausted and, as such, is ongoing.



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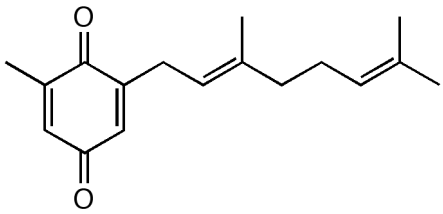

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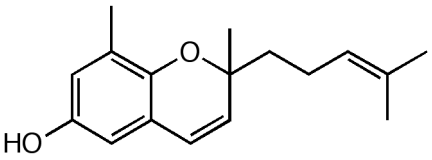

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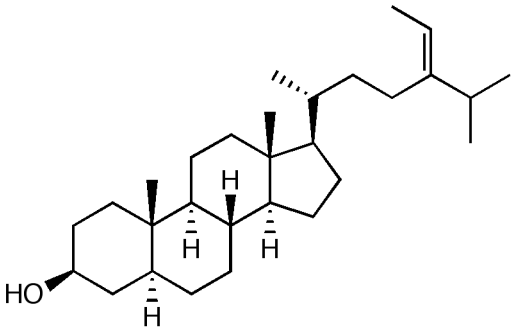

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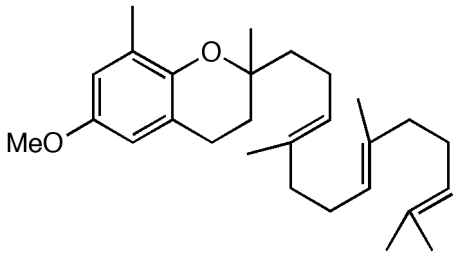

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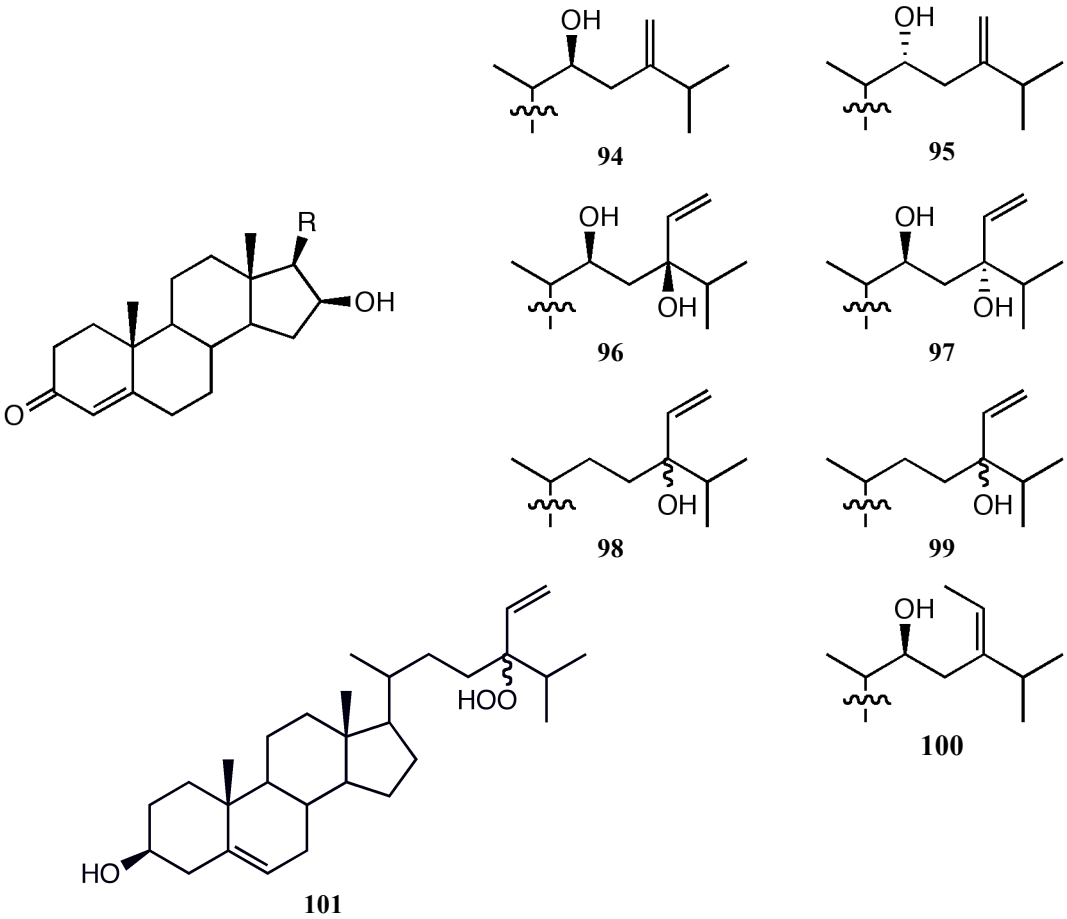
As the putative ancestor of the genus, *C. xiphocarpa* was expected to possess only the more common and wide-spread *Cystophora* compounds, such as phloroglucinols and tocotrienols. To date no secondary metabolites are reported from the species and it was decided to investigate the chemistry of specimens collected in Tasmania. GC-MS of the methyl esters of a transesterified triacylglycerol isolated identified at least thirteen acyl chains, 14:0, 16:3*n*-6,

16:0, 18:3*n*-6, 18:4*n*-3, 18:2*n*-6, 18:3*n*-3, 20:4*n*-6, 20:5*n*-3, 20:3*n*-6, 20:4*n*-3, 20:2 and 20:3*n*-3, indicating that the triacylglycerol mixture is comprised of at least five different compounds.

Cystophora xiphocarpa also yielded a series of eight polyoxygenated steroids (**94**–**101**), which includes three pairs of diastereomers, as well as a phaeophytin (**102**). Investigation of the stereochemistry of the isolated steroids included the derivatisation of compound **94** using phosgene to form a cyclic carbonate (**106**), molecular modelling and coupling constant (*J*) analysis of each compound. As a result, the stereochemistry of only one pair of diastereomers remains undefined. Unfortunately these steroids decomposed before anticancer bioassay data could be obtained. The biosynthesis of steroids is also discussed and a biosynthetic pathway of each of the steroids identified during this research is proposed.



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