

CYTOTOXIC COMPOUNDS FROM BRITISH MARINE ALGAE

G. BLUNDEN¹, N. A. S. SHOEIB^{2,3} and C. W. WRIGHT²

¹School of Pharmacy, University of Portsmouth, Portsmouth, P01 2DT;

²School of Pharmacy, University of Bradford, Bradford, BD7 1DP;

³Pharmacognosy Department, Faculty of Pharmacy, Tanta University, Tanta, Egypt



INTRODUCTION

A number of compounds from marine algae have been reported to have antitumour and cytotoxic properties. Ma'iliohydrin (**1**), a tribrominated chamigrene, was isolated from an undetermined species of *Laurencia* from the Philippines. The compound exhibited cytotoxicity in the U. S. National Cancer Institute (NCI) 60-cell line human tumour screen and displayed especially potent activity against the NCI/AD-R-RES breast cancer cell line ¹.

Jania rubens yielded a cytotoxic steroid, 16 β -hydroxy-5 α -cholestane-3, 6-dione (**2**) with an ID50 value of 0.5 μ g/ml against KB (human nasopharynx carcinoma) cells ². Seven oxygenated desmosterols (for example **3**) have been isolated from the red alga *Galaxaura marginata*, all of which were shown to exhibit significant cytotoxicity to several cancer cell lines ³.

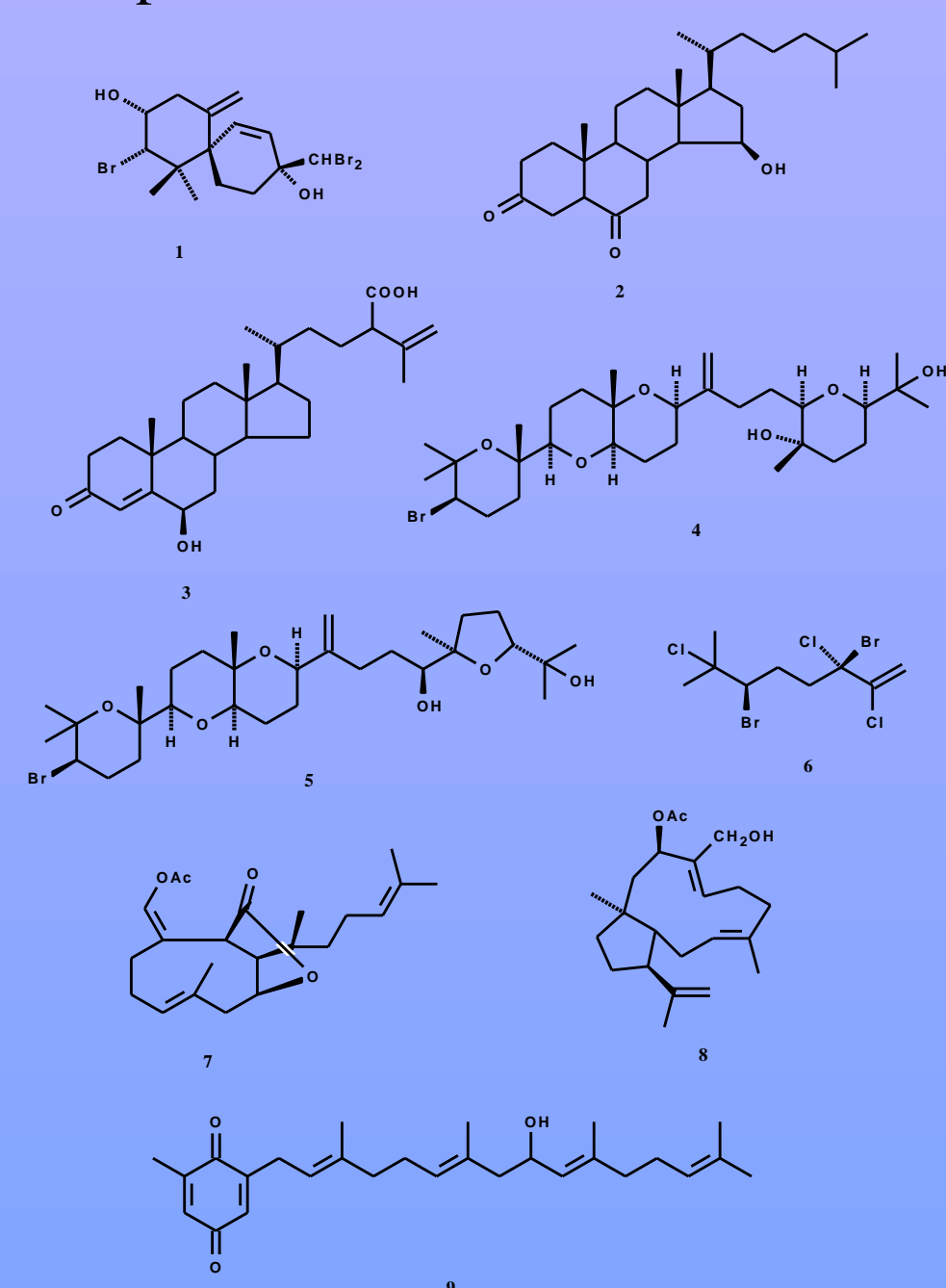
Two squalene-derived brominated triterpenes, isodehydrothyriferol (**4**) and 10-epidehydrothyriferol (**5**), isolated from *Laurencia viridis*, were shown to have potent cytotoxic activities against a number of cancer cell lines ⁴.

A polyhalogenated acyclic monoterpene, halomon (**6**), was obtained from the red alga, *Portieria hornemannii* ⁵. The compound exhibited highly differentiated cytotoxicity to the NCI *in vitro* human tumour cell line screening panel and was selected for preliminary drug development.

Diterpenoids and a novel xenicane-type diterpene (**7**) with cytotoxic activities against a number of mammalian cell lines ⁶ were isolated from the brown alga *Dilophus ligulatus*. One diterpene (**8**), isolated from an unidentified *Dictyota* species, exhibited significant cytotoxicity against leukaemia P-388 cells ⁷.

Hydroxysargaquinone (**9**) was isolated from *Sargassum tortile* and was demonstrated to have significant cytotoxicity towards P-388 cells ⁸.

In this current programme, extracts of marine algal species are being screened for cytotoxicity and the extracts are being fractionated in order to isolate and characterize the active compounds. Some of the data obtained are presented here.



MATERIALS AND METHODS

Marine algal species were collected from Kimmeridge, Dorset, U. K., Bembridge, Isle of Wight, U. K. and Finavarra, Co. Clare, Republic of Ireland. The seaweeds were dried at 60 $^{\circ}$ C and powdered. Each species (5 g) was extracted at room temperature with 3 x 100 ml methanol and the combined extracts were concentrated to dryness under reduced pressure (less than 40 $^{\circ}$), then dried under nitrogen and vacuum at room temperature. Serial two-fold dilutions of the dried extract, starting from 500 μ g/ml, were prepared, dissolved in DMSO and diluted in RPMI 1640 medium, so that the DMSO concentration did not exceed 0.1%. DMSO was found to be non-toxic at this concentration. The extracts were assessed using the *in vitro* 96-hour continuous exposure MTT assay, which involves the metabolism of MTT to the coloured product formazan by mitochondrial enzymes in living cells ⁹⁻¹¹.

For the active species, the methanol extracts were partitioned between chloroform and water. The chloroform fractions were dried by shaking with anhydrous sodium sulphate, filtered, concentrated under reduced pressure and dried under nitrogen before storage at 4 $^{\circ}$ C until tested for biological activity. The aqueous fractions were freeze dried and also stored at 4 $^{\circ}$ C until tested.

Chemosensitivity was expressed as an IC₅₀ value. 5-Fluorouracil was used as a positive control.

RESULTS

The cytotoxic activities of the methanol extracts of the marine algae tested are presented in Tables 1-3.

Table 1. Cytotoxic activities of green algae tested

Species	Cytotoxicity (IC ₅₀) μ g/ml
<i>Codium fragile</i> subsp. <i>tomentosoides</i>	134
<i>Enteromorpha intestinalis</i>	>500
<i>Ulva lactuca</i>	288

Table 2. Cytotoxic activities of brown algae tested

Species	Cytotoxicity (IC ₅₀) μ g/ml	Species	Cytotoxicity (IC ₅₀) μ g/ml
<i>Spongonema tomentosum</i>	>500	<i>Padina pavonia</i>	<500
<i>Colpomenia peregrina</i>	>500	<i>Ascophyllum nodosum</i>	356
<i>Desmarestia aculeata</i>	>500	<i>Fucus serratus</i>	380
<i>Laminaria digitata</i>	>500	<i>F. spiralis</i>	404
<i>L. hyperborea</i>	358	<i>F. vesiculosus</i>	259
<i>L. saccharina</i>	>500	<i>Pelvetia canaliculata</i>	190
<i>Saccorhiza polyschides</i>	168	<i>Sargassum muticum</i>	194
<i>Dictyota dichotoma</i>	260		

Table 3. Cytotoxic activities of red algae tested

Species	Cytotoxicity (IC ₅₀) μ g/ml	Species	Cytotoxicity (IC ₅₀) μ g/ml
<i>Furcellaria lumbricalis</i>	>500	<i>Chylocladia verticillata</i>	>500
<i>Calliblepharis jubata</i>	>500	<i>Ceramium rubrum</i>	>500
<i>Plocamium cartilagineum</i>	148	<i>Griffithsia flosculosa</i>	358
<i>Chondrus crispus</i>	>500	<i>Cryptopleura ramosa</i>	>500
<i>Dilsea carnosa</i>	486	<i>Halopitys incurvus</i>	328
<i>Callophyllis flabellata</i>	>500	<i>Polysiphonia lanosa</i>	40
<i>Palmaria palmata</i>	>500	<i>Porphyra umbilicalis</i>	>500

The activities of *Codium fragile* subsp. *tomentosoides*, *Cystoseira baccata*, *Plocamium cartilagineum*, *Polysiphonia lanosa* and *Saccorhiza polyschides* were considered suitably high to warrant further investigation. The methanol extracts of each of these species were fractionated between chloroform and water and each fraction was tested for cytotoxicity, as before. All the chloroform fractions were shown to be highly active (Table 4)

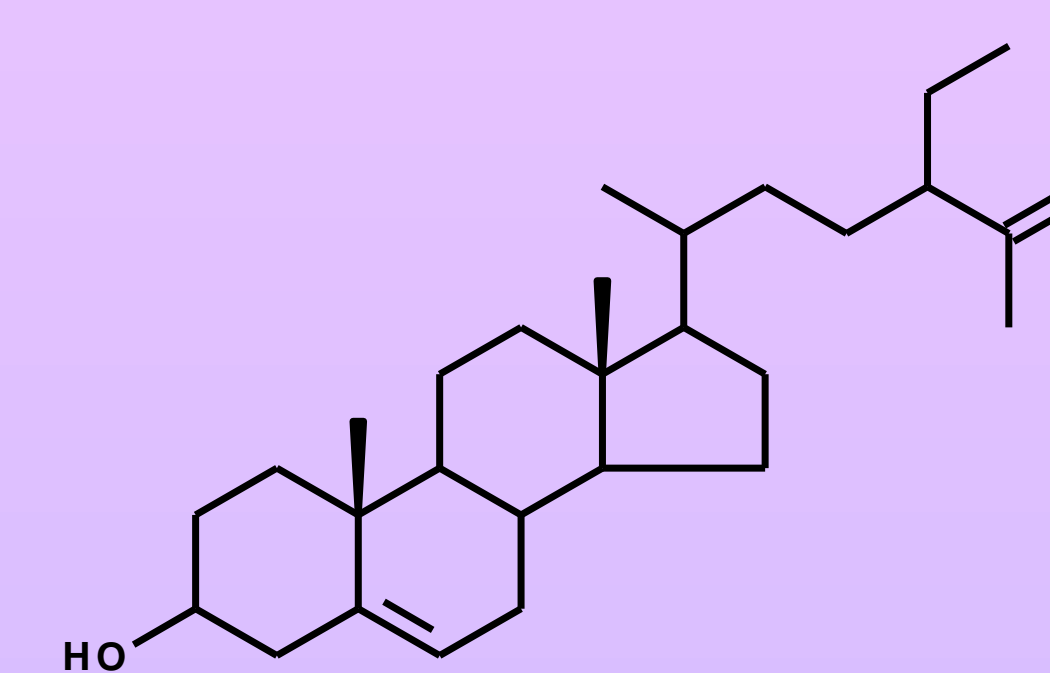
Table 4. Cytotoxic activities of the chloroform fractions of the active species

Species	Cytotoxicity IC ₅₀ \pm SD (μ g/ml)
<i>Codium fragile</i> subsp. <i>tomentosoides</i>	5.18 \pm 0.87
<i>Cystoseira baccata</i>	22.5 \pm 1.45
<i>Saccorhiza polyschides</i>	39.7 \pm 2.34
<i>Polysiphonia lanosa</i>	4.58 \pm 0.79
<i>Plocamium cartilagineum</i>	5.56 \pm 0.93

Codium fragile subsp. *tomentosoides*

Bioassay-guided fractionation of the chloroform extract of *Codium fragile* subsp. *tomentosoides* led to the isolation of clerosterol (**10**), the IC₅₀ value against DLD-1 cells for which was 5.56 \pm 0.48 μ g/ml. Two pigments isolated were also shown to have IC₅₀ values of 5.63 and 4.44 μ g/ml, respectively. However, these pigments were not pure enough to enable determination of their structures and the amounts left after cytotoxicity experiments were too small to allow further purification.

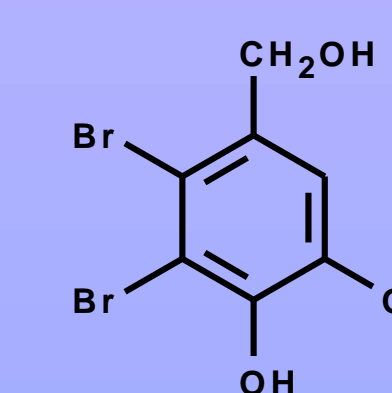
Clerosterol has been isolated previously from *Codium fragile* and other species of *Codium*. This sterol has also been shown to have *in vitro* cytotoxic activity against P-388 and A-549 cells, with IC50 values of 1.7 and 0.3 μ g/ml, respectively¹².



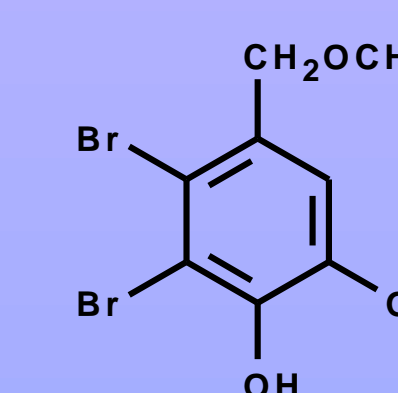
Clerosterol

Polysiphonia lanosa

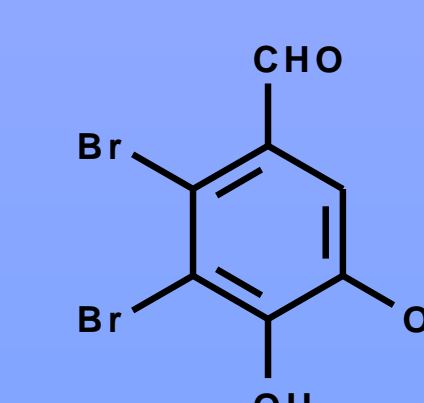
Primarily from gas/liquid chromatography/mass spectrometric data, the major cytotoxic components of the chloroform fraction of *Polysiphonia lanosa* were proved to be the bromophenols lanosol (**11**; IC₅₀ 18.27 \pm 0.94), the aldehyde of lanosol (**12**; IC₅₀ 30.94 \pm 2.7), the *n*-propyl ether of lanosol (**13**; IC₅₀ 12.35 \pm 1.14) and the methyl ether of lanosol (**14**; IC₅₀ 14.56 \pm 3.07), all of which have been reported previously as constituents of the alga¹³. For more detailed studies of these compounds, they were synthesised and a number of derivatives were also prepared. The cytotoxicities of some of these synthetic compounds were significantly greater than those of the compounds isolated from the alga¹⁴.



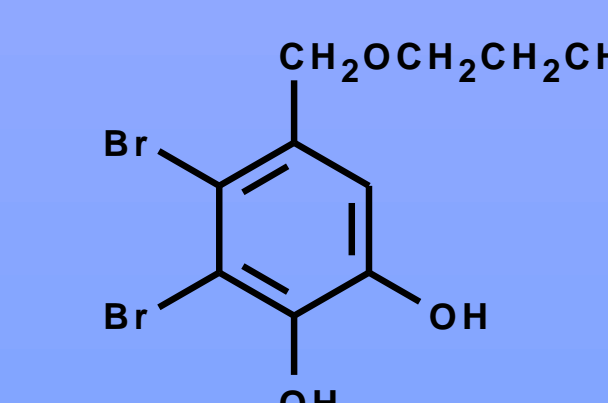
Lanosol (11)



Methyl ether of Lanosol (14)



Aldehyde of Lanosol (12)



N-Propylether of Lanosol (13)

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