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Seasonal Variation in Bromophenol Content of *Polysiphonia lanosa*

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The bromophenol content of *Polysiphonia lanosa*, determined by GLC-MS, was shown to vary considerably between late spring and early autumn. The lowest content was found in material collected in early May and then the levels rose until a peak was reached in July. When the extracts were tested for cytotoxicity against DLD-1 cells, a direct correlation was shown between cytotoxicity and bromophenol abundance, confirming that these compounds are the ones responsible for the cytotoxicity of the species.

Keywords: *Polysiphonia lanosa*, Rhodomelaceae, bromophenols, cytotoxicity, seasonal variation.

The isolation and characterization of brominated phenols from the red alga *Polysiphonia lanosa* (L.) Tandy [*Vertebrata lanosa* (L.) T. Christensen] (Rhodomelaceae) has been reported [1, 2]; the compounds identified were 2, 3-dibromo-5-hydroxy-benzyl-1', 4-disulphate dipotassium salt, 3-bromo-4, 5-dihydroxybenzaldehyde, along with its methyl and ethyl ethers, 3-bromo-4, 5-dihydroxybenzyl alcohol, 2, 3-dibromo-4, 5-dihydroxybenzyl alcohol (lanosol), as well as its methyl, ethyl and *n*-propyl ethers, 2, 3-dibromo-4, 5-dihydroxybenzaldehyde (the aldehyde of lanosol), 3, 5-dibromo-4-hydroxybenzyl alcohol and 2, 3, 6-tribromo-4, 5-dihydroxybenzyl alcohol. Later, Glombitza *et al* [3] isolated two further bromophenols, rhodomelol and methylrhodomelol.

Extracts of *P. lanosa* have been reported to have antimicrobial [2, 3] and cytotoxic properties [4]; bromophenols were shown to be the active compounds. Several investigators have recorded pronounced seasonal changes in the levels of antimicrobial activity of seaweed extracts [for example, 5-8]. Tariq *et al* [8] investigated *P. lanosa* and stated that a sample collected in November had a significantly higher level of antifungal activity than the samples collected at other times of the year. In our present study, we have examined seasonal variations in the bromophenol content of *P. lanosa* by collecting and analyzing samples collected at different times of the year. These results were correlated with the cytotoxicity of the extracts.

In previous work, from GLC-MS data, the major bromophenols identified by us in the most cytotoxic fractions of the chloroform extracts of *P. lanosa* were lanosol, methyl, ethyl and *n*-propyl ethers of lanosol, and the aldehyde of lanosol, although the ethylether appeared to be an artifact arising from the fractionation procedure [4]. In this present study, the four major bromophenols detected in the chloroform fractions of the methanol extract were lanosol, the aldehyde of lanosol, and the *n*-propyl and methylethers of lanosol. This was the case with all the samples collected. As a parameter representing bromophenol abundance, the proportions of each of these compounds present in the extracts of the different collections of *P. lanosa* were compared using the area under the GLC peaks. The relative

quantity of each of the major bromophenols present in the chloroform fractions of the tested samples is represented in Figure 1. The methylether of lanosol was the most abundant bromophenol in all the collections, except that made at the end of May, in which lanosol predominated.

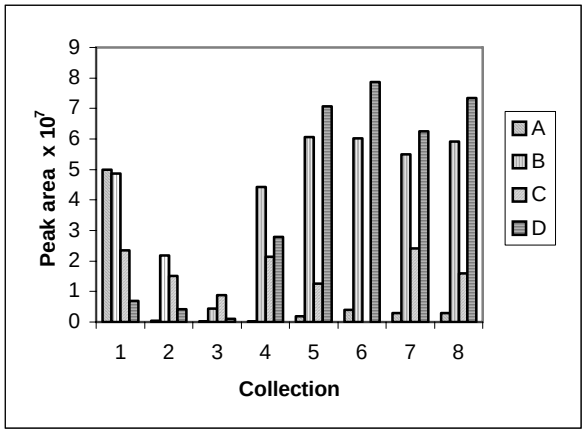

Figure 1: *Polysiphonia lanosa*: Abundance of individual bromophenols (represented as peak area) in the chloroform fractions of methanol extracts of collections made between late spring and early autumn.
Date of collection: 1, July 1999; 2, June 2000; 3, Early May 2001; 4, Late May 2001; 5, June 2001; 6, July 2001; 7, August 2001; 8, Early October 2001.
Compounds: A, aldehyde of lanosol; B, lanosol; C, *n*-propyl ether of lanosol; D, methyl ether of lanosol.

The highest contents of bromophenols were found in the two July collections; there was no significant difference between the July 1999 and July 2001 samples. The lowest bromophenol content was recorded for the algal material collected in early May, whereas in the collection made later in the month, the content was noticeably higher and further increases were recorded for the June and July samples. The bromophenol levels in the August and October samples were noticeably lower than in the July collection (Table 1).

Each of the *P. lanosa* samples was composed of numerous specimens taken from a minimum of twenty-five *Ascophyllum nodosum* plants. After drying, the *P. lanosa* specimens were mixed well and so each sample taken was deemed to be representative of that time of collection.

The chloroform fractions of the different collections of *P. lanosa* were tested for cytotoxicity against DLD-1 cells to study the effect of seasonal variation on cytotoxic activity. The results are presented in Table 1. Cytotoxic activity was found to be directly proportional to the bromophenol abundance. As bromophenol content increased, so did cytotoxicity. The most active collections were those made in July and the least active sample was that collected in early May.

Table 1: *Polysiphonia lanosa*: Cytotoxic activity against DLD-1 cells of the chloroform fractions of methanolic extracts of samples collected at different times of the year.

Date of collection	Cytotoxic activity (IC_{50}±SD (μg/ml) n=3
July 1999	4.58 ± 0.79
June 2000	15.01 ± 1.73
Early May 2001	18.23 ± 1.98
Late May 2001	11.37 ± 0.79
June 2001	7.79 ± 1.26
July 2001	4.18 ± 0.42
August 2001	7.49 ± 0.40
Early October 2001	6.53 ± 1.40

P. lanosa does not appear to be subjected to extensive grazing and is generally free from epibiont growth; this is possibly due, at least in part, to the bromophenols. The risk of attack by grazers and the growth of epibionts would be expected to be greatest in the spring and summer and this may well be the reason for the increased levels of bromophenols during this period.

Experimental

Algal material: Samples of *Polysiphonia.lanosa* were collected from Kimmeridge Bay, Dorset, UK at different times of the year (see Figure 1 and Table 1).

Each algal sample was comprised of numerous *P. lanosa* specimens taken from a minimum of 25 *Ascophyllum nodosum* plants. The algal material was dried as quickly as possible at 50°C, powdered and mixed well. A voucher sample of *P. lanosa* is deposited with the Hampshire County Council Museums Service, Winchester, Hampshire, UK (Index Herbariorum code HCMS; accession number Bi 2000. 16. 271).

Extraction and fractionation: Each dried, powdered seaweed sample (5 g) was extracted successively with *n*-hexane and methanol (x 3) at room temperature. The combined methanol extracts were concentrated to dryness under reduced pressure and the residue fractionated between chloroform and water. The chloroform fraction was concentrated to dryness and kept at 4°C until ready for use.

GLC-MS: The chloroform fractions were analyzed by GLC-MS. Identification of the bromophenols was made from the mass spectra of the trimethylsilyl derivatives after GLC [4, 9]. Estimation of the content of each bromophenol was based on the area under the appropriate GLC peak. The performance of the instrument was validated prior to each analysis using a series of reference *n*-alkanes.

In-vitro cytotoxicity assay: The extracts were tested for cytotoxicity against human colon cell line DLD-1 using the MTT colorimetric assay [10]. This procedure is described in an earlier publication [4].

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