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SIMPLE BROMINATED PHENOLS IN RED ALGAE

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Key Word Index—Rhodophyta; Rhodomelaceae; red algae; bromophenols.

Abstract—By using TLC and GLC–MS techniques, 23 species of red algae, representing nine orders, were analyzed for the presence of simple bromophenols. Ten bromophenols were detected, five of which might be artefacts. Furthermore, lanosol was identified in sea water from the *Polysiphonia Brodiaei*—zone. Bromophenols were detected in species from the families Ceramiaceae, Delesseriaceae, Bonnemaisoniaceae, Rhodophyllaceae, Corallinaceae and Rhodomelaceae.

INTRODUCTION

A NUMBER of bromine-containing organic compounds have been described from the Rhodomelaceae (Rhodophycophyta); these include simple brominated phenols,[1–7] brominated heterocyclic compounds[8–10] and the hitherto poorly known red pigment floridorubin.[5] No bromine-containing compounds have as yet been isolated from other red algal families, although Kylin[11] suspected covalently-bound bromide on the basis of colour reactions and solubility in a number of non-rhodomelaceous algae, including *Antithamnion*, *Antithamnionella*, *Ceramium*, *Trailliella* and *Bonnemaisonia*.

The occurrence and distribution of simple brominated phenols have been investigated through the use of their colour reaction with $FeCl_3$[12] and their chromatographic behaviour coupled with various colour reagents.[13] However, since only a small selection of algae have been investigated for simple bromophenols, a survey covering a large number of red algae belonging to various families was initiated to determine a more exact distribution of these compounds. In addition, the various extracts from these algae were tested for their colour reactions with several spray reagents and chromatographic behaviour on paper and thin-layers. In this report, the findings of previous workers have been summarized and the results of the present survey are discussed.

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RESULTS

Early in this survey, the sensitivity of the GLC–MS technique was determined, as many of the algae examined were available only in very small amounts. It was found that a ninth of the total volume of extract made from 0·8 g of dry algal material sufficed for the detection of five bromophenols in *P. urceolata*, indicating that 90 mg of algal material (dry weight) is sufficient for GLC–MS analysis of the main phenolic compounds. During this investigation the same volume of hot water phase (Fig. 1) was used for the GLC–MS analysis, of all algae, except for the Porphyridiales and Goniotrichales where the whole extract was used. This ensured that in these species no bromophenols were overlooked on account of the small amounts of algal material available for analysis.

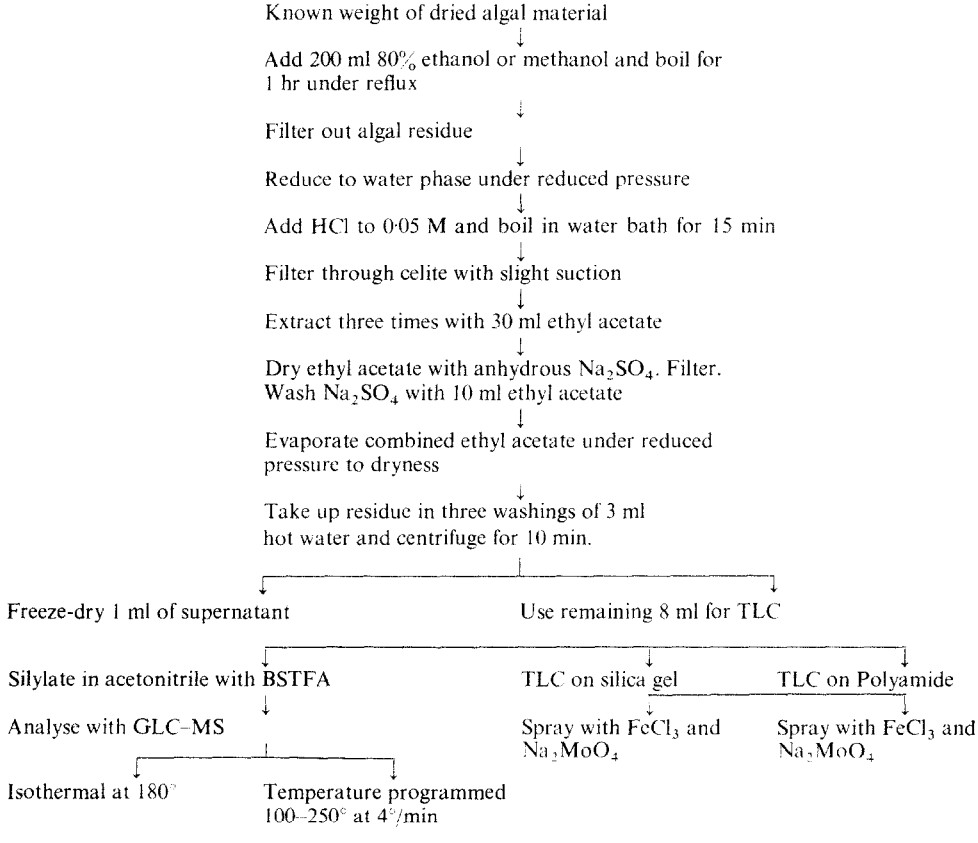

FIG. 1. FLOW DIAGRAM OF METHOD USED FOR ANALYSES OF SIMPLE BROMINATED PHENOLS IN 23 SPECIES OF RED ALGAE.

Comparison of bromophenols from fresh and dried material of P. Brodiaei

The $FeCl_3$-reactive components of the freshly extracted and dried algal material were compared using ascending paper chromatography and examined under UV light and their fluorescence colour, R_f values and colour reactions with sodium molybdate were compared. In both extracts the same two main phenols **6** and **6**m and two minor components **4** and **3** were observed. An identical distribution of these four bromophenols was apparent in both extracts using TLC.[2] Using the more sensitive combined GLC–MS technique, the same four bromophenols were detected in the same proportions in the two extracts but

two additional bromophenols **2** and **5**, not detected by PC or TLC, were found in both extracts. From these results, it is apparent that the drying of the algal material does not affect these compounds and since drying provided a convenient method of treating the material during field collecting, it was adopted in the subsequent survey of the 23 species of algae used.

Mass spectra and the identification of the bromophenols

All initial identifications of the bromophenols were made from the mass spectra of the TMS derivatives after GLC. With the exclusion of some bromophenols, which will be described in a later paper, a total of 10 simple bromophenols were found, of which five might be artefacts. From the mass spectra the number of bromine atoms in the molecule, the number of silyl-ether (–OH) groups and the molecular weights can be readily determined (Table 2). The fragmentation pattern of all the compounds are very similar, the molecular ion (**M**) or the ion **M**–Me generally forming the base peak.[7]

Further identifications of ring substituents were made on the basis of the Na_2MoO_4 colour reaction for catechols[15] and by comparison with previously described bromophenols.[2–4,7,14] These showed that *o*-dihydroxy groups were invariably in the 4,5-positions.[3,4,6,14] Single hydroxy-methylene groups are usually in the 4-positions.[2] Similarly where two bromosubstituents were found, these occupy positions 2,3-[4,7,14] except in 3,5-dibromo-*p*-hydroxybenzyl alcohol,[2] while single bromosubstituents were in position 3-.[6] The orientation of the substituents was based on previously published data.[2–4,7,14] The bromophenols and their known distribution are presented in Table 1. Compounds **1**m, **2**m, **2**e, **6**m and **6**e might be artefacts produced during the extraction procedure. Stoffelen *et al.*[7] found that when using a non-aqueous extracting medium (ethyl acetate), these ethers were absent.

GLC retention times

Using the described GLC system, reproducibility of retention time for each compound was high and identifications could be made on the basis of isothermal and temperature-programmed retention times provided other compounds with similar retention characteristics did not mask the compound peaks.

NMR and IR spectroscopy

Compound **6** showed the presence of a single aromatic proton ($\delta = 7{\cdot}04$), a methylene group of a benzyl alcohol ($\delta = 4{\cdot}51$) and three hydroxyl protons (broad peak at $\delta = 3{\cdot}8$ with slight peaks at $\delta = 3{\cdot}81$, 3·73, and 3·62). The hydroxyl proton peaks were intensified by the addition of a drop of pyridine or chloroform to the deuterated acetonitrile but resulted in no significant shift in their positions.

A KBr disc IR spectrum of compound **6** indicated the presence of a hydroxy group (3350 cm^{-1}), a benzene ring (3050, 1590, 1575 and 890 cm^{-1}), a benzylic methylene group (3010, 2910 and 1470 cm^{-1}) and a C–Br group (560 cm^{-1}). Good agreement of the IR spectrum was found with the sulphonated compound **6**[4] except that there were no indications of the absorption bands due to the sulphate esters at 1250 and 1030 cm^{-1}.

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TABLE 1. RED ALGAE INVESTIGATED, SOURCES AND BROMOPHENOLS PRESENT

Order, family and species	Source	Dry weight (g)	Bromophenols identified
Bangiales			
Bangiaceae			
Porphyra umbilicalis (L.) Kütz	I	10·1	—
Porphyridiales			
Porphyridiaceae			
Rhodosorus marinus Geitler	L	0·5	—
Goniotrichales			
Goniotrichaceae			
Asterocytis ramosa (Thwaites) Gobi et Schm.	L	0·8	—
Goniotrichum alsidii (Zanard.) Howe	L	0·2	—
Nemalionales			
Helminthocladiaceae			
Nemalion multifidum (Weber et Mohr) J. Ag.	K	3·1	—
Rhodymeniales			
Rhodymeniaceae			
Rhodymenia palmata (L.) Grev.	I	5·9	—
Gigartinales			
Rhodophyllaceae			
Cystoclonium purpureum (Huds.) Batt.	S	1·9	**5**
Cryptonemiales			
Dumontiaceae			
Dilsea carnosa (Schmidel) Kuntze	S	18·9	—
Corallinaceae			
Corallina officinalis L.	K	4·0	**6**
Bonnemaisoniales			
Bonnemaisoniaceae			
Trailliella intricata Batt.	K	5·1	—
Falkenbergia rufolanosa (Harvey) Schmitz	M	20·1	—
Ceramiales			
Rhodomelaceae*			
Polysiphonia Brodiaei (Dillw.) Grev.	K	4·5	**2**–**5** **6**,[3] **6m**
P. urseolata (Dillw.) Grev.	I	0·8	**3**–**6** **6m**
P. nigrescens (Huds.) Grev.	I	2·1	**2**, **3**, **6**,[3] **6m**
Rhodomela confervoides (Huds.) Silva	I	7·1	**2**,[2] **5**, **6**,[2,3] **6m**
Odonthalia dentata (L.) Lyngb.	I	4·8	**1m**, **2**,[2] **2m**, **2e**, **3**, **4**, **5**, **6**,[2] **6m**, **6e**
Laurencia pinnatifida (Huds.) Lamour.	I	3·7	—
Dasyaceae			
Heterosiphonia plumosa (Ellis) Batt.	S	3·9	—
Dasya baillouviana (Gmel) Mont	K	2·9	—
Delesseriaceae			
Phycodrys rubens (Huds.) Batt.	I	3·6	**6m**
Delesseria sanguinea (Huds.) Lamour	I	6·5	—
Ceramiaceae			
Antithamnion plumula (Ellis) Thur.	S	1·9	**6**, **6m**
Ceramium rubrum (Huds.) Ag.	S	6·2	**6**, **6m**

* Previously investigated species are *Polysiphonia lanosa* **3**,[7] **4**,[7] **6**,[4,7] **6m**,[7] **6e**[7] *P. morrowii* **3**,[6] *P. nigra* **6**,[3] *P. elongata* **6**,[3] *P. fruticulosa* **6**,[3] *Rhodomela larix* **5**, **6m**,[14] and *Odonthalia corymbifera* **6m**.[14]

K—collected near the Kristineberg Zoological Station.
I—collected by scuba diving at Islandsberg, south of the station.
S—collected by dredging at Smedjan, west of the station.
M—collected near Banyuls-sur-Mer, Mediterranean Sea.
L—cultivated axenically in enriched sea water.[16]

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TABLE 2. PROPOSED STRUCTURES AND MS OF THE TMS DERIVATIVES OF THE SIMPLE MONO- AND DI-BROMOPHENOLS IDENTIFIED

Proposed structure		Significant peaks at MS
	**1**m	(**M**) 288, 273, 257, 242, 209, 147, 137, 73
	**2** R = H	(**M**) 424, 409, 345, 335, 257, 147, 137, 73
	2m R = Me	(**M**) 366, 351, 335, 320, 287, 272, 257, 242, 147, 137, 73
	2e R = Et	(**M**) 380, 365, 335, 321, 301, 257, 242, 147, 137, 73
	**3**	(**M**) 360, 345, 272, 257, 178, 147, 137, 73
	**4**	(**M**) 434, 419, 355, 345, 331, 257, 147, 137, 73
	**5**	(**M**) 438, 423, 378, 344, 335, 257, 147, 137, 73
	**6** R = H	(**M**) 512, 497, 433, 423, 335, 409, 257, 178, 147, 137, 73
	6m R = Me	(**M**) 454, 439, 423, 375, 335, 257, 178, 147, 137, 73
	6e R = Et	(**M**) 468, 423, 389, 345, 335, 257, 178, 147, 137, 73

Colour reactions with $FeCl_3$ and Na_2MoO_4

Since some of the initial distribution data of brominated phenols were based on their colour reaction with $FeCl_3$,[12] extracts from 23 algal species were spotted onto paper and sprayed with 3% $FeCl_3$ in order to test the correlation between the colour reaction and the bromophenol content. Of the 23 algal extracts, those from *Rhodomela*, *Odonthalia*, *Polysiphonia Brodiaei*, *P. urceolata* and *P. nigrescens* gave distinct purple-grey spots while those of *Ceramium rubrum*, *Antithamnion plumula*, *Phycodrys rubens*, *Trailliella intricata*, *Falkenbergia rufolanosa*, *Delesseria sanguinea*, *Cystoclonium purpureum*, *Nemalion multifidum*, *Rhodymenia palmata*, *Porphyra umbilicalis*, *Corallina officinalis*, *Rhodosorus marinus*, *Asterocytis ramosa* and *Goniotrichum alsidii* gave no colour reaction at all. *Dasya baillouviana* and *Heterosiphonia plumosa* extracts produced faint green spots, the *Laurencia pinnatifida* extract a faint red spot and that of *Dilsea carnosa* a faint orange spot. These colour reactions indicate that other $FeCl_3$-reactive compounds are present. Furthermore, the sensitivity of the $FeCl_3$ spray is such that only those species with a large concentration of bromophenols resulted in a distinct purple spot while those species with lower concentrations (*Corallina*, *Phycodrys*, *Antithamnion* and *Ceramium*) gave no colour reaction.

Chromatography of the various algal extracts on polyamide thin layers was followed by spraying with sodium molybdate. Immediate yellow spots were formed with three compounds (R_f 0·13, 0·22 and 0·35) in the extracts of *Odonthalia*, *Rhodomela* and *Polysiphonia*. No other colours were observed. The three compounds of the above R_f value correspond to compounds **6**m, **5** and **6** respectively, the most abundant bromophenols in the rhodomelaceous algae. Since no other colours were produced, it appears that this spray, although quite specific for catechols, is too insensitive and its application is limited.

Brominated phenol in sea water from the "Polysiphonia Brodiaei-*Zone*"

By injecting 8 μl of the silylated extract of this sea water on to the column, two bromophenols (**6** and **6e**) were found.

DISCUSSION

Of the red algae, members of the Rhodomelaceae are particularly rich in bromine[12,17] and it is not surprising that simple brominated phenols were first reported from this algal family.[2] Since that time a number of simple brominated phenols have been found in the Rhodomelaceae (Table 1) in medium to high concn; lanosol* constitutes between 1–5% of the dry weight of *Polysiphonia lanosa*[4] and 0·5% in *Polysiphonia Brodiaei* (present study). Based on the fresh-weight of the algae, 3,5-dibromo-*p*-hydroxybenzyl alcohol constitutes 0·024 and 0·003% of *Odonthalia dentat* and *Rhodomela confervoides* respectively.[3] There are no previous records of simple brominated phenols from non-rhodomelaceous algae and the concentrations of those found in this survey are much smaller than of those found in the Rhodomelaceae.

No bromophenols were found in members of Porphyridiales, Bangiales, Goniotrichales, Nemalionales, Rhodymeniales and the family Dasyaceae. *Delesseria sanguinea* contained no bromophenols but in *Phycodrys rubens* the ether **6m** was detected. Corallinaceae and Ceramiaceae contained **6** and Ceramiaceae also the ether **6m**. In the representative of Gigartinales (*Cystoclonium purpureum*) only the aldehyde **5** was detected. *Trailliella intricata* and *Falkenberga rufolanosa* appeared to be the most interesting algae containing several bromophenols not reported before. These algae are now under further investigation. In addition it is interesting to note the absence of simple bromophenols in *Laurencia pinnatifida*, belonging to a genus where a number of brominated heterocyclic compounds have been found.[9,10] However, the possibility of an annual fluctuation in the content of bromophenols requires investigation.

Compound **6** (lanosol) appears to be the most abundant and widely distributed of the bromophenols. **2**, **4** and **6** are known to occur as the sulphate esters.[3,4,13,18] The presence of the sulphate esters appears to decrease the antibiotic activity[7,19,20] when compared to the desulphated compounds.[21,22]

The metabolic role of the brominated phenols is unknown, but in addition to other non-brominated phenols in low concentrations (0·2 mmol/l.), they stimulate the growth of some red algae.[23] **6** and **3** are toxic to unicellular marine algae at levels below 0·3 mmol/l.[19] Bromophenols are indicated to induce some morphogenetic changes in *Ulva*.[24] The occur-

* The positions of the sulphate esters of this compound have recently been questioned;[6] these authors maintain that the sulphates occur in the 1,4-positions.

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[23] FRIES, L. (1973) *Experientia.* **29**, 1436.
[24] PROVASOLI, L. and PINTER, J. J. (1966) *11th Pac. Sci. Congr., Proc. Abstr. Pap. Fish.* **7**, 23.

rence of lanosol in sea water raises some interesting ecological questions concerning the antagonistic and growth stimulating effects on diverse algal groups. The influence of different bromophenols on algal growth under axenic conditions is presently under investigation.

EXPERIMENTAL

Collection and initial extraction. Approximately 1 k of *Polysiphonia Brodiaei* (Dillw.) Spreng. was collected from the intertidal zone in August 1972 in the vicinity of the Kristineberg Zoological Station on the west coast of Sweden. Five-hundred g were rinsed in fresh water, placed into aluminium bags and oven dried at 45° for 48 hr. The remaining portion was brought back to the laboratory and deep-frozen. Both the dried and deep-frozen tissue was subsequently analyzed by the following procedure and the results compared. The algal tissue was placed into 2 l. boiling 90% aq. MeOH and heated under reflux for 3 hr, after which the MeOH was poured off. The extraction was repeated twice using 80% MeOH. These fractions were combined, filtered through Munktell paper (A3-90-700) and freed from MeOH by rotary evaporation. The aqueous solution was subsequently filtered through Hyflow celite under slight suction, the celite being prewashed with 2 l. boiling distilled H_2O. Ester sulphates were removed by making the aq. soln up to 0·05 M HCl and heating for 10 min. The resulting solution was partitioned with EtOAc until the EtOAc phase was colourless. These fractions were pooled, frozen to remove traces of water and an approximately eq. vol. of petrol (b.p. 40–60°) was added. The resultant crystals were dissolved in a small vol. of H_2O.

Subsequent analytical procedures. Subsequent collections of algal material were rinsed briefly in fresh water and oven dried at 45° for 48 hr. For the survey of the distribution of simple bromophenols, 23 species of red algae were collected, representing nine rhodophycean orders. A list of the algae and relevant collection data are provided in Table 1. Dried pulverised, algal material was subsequently treated as indicated above.

GC–MS. The freeze-dried samples were silylated by adding 20 μl of acetonitrile and 20 μl of BSTFA (*N,O*-bis-TMS-trifluoroacetamide containing 1% TMCS as catalyst, Pierce Chemical Co.) at 40° for 5 min. Analyses of the TMS derivatives were carried out on a Varian Aerograph model 2100–20 GS equipped with flame ionization detectors and a 1,2 m, 4 mm i.d., silanized glass column packed with 3% (w/w) SE 30 on a 80/100 mesh acid washed, DMCS-treated chromosorb W. A carrier gas (97% N_2 and 3% H_2) flow rate of 25 ml/min was maintained. Injector and detector temp. were held constant at 250 and 280° respectively. Retention times were determined isothermally at 180° and temp. programmed from 100–250° or 50–250° at 4°/min. All syringes were dried at 60° and kept over P_2O_5. The MS were determined with a Varian Mat 111 (Gnom) MS coupled to a V.A.G. Chromatograph equipped with a 1,2 mm, 2 mm i.d. silanized glass column. He was used as carrier gas at a flow rate of 25 ml/min.

NMR and IR analysis. NMR analyses of component **6** were carried out in CD_3CN on a Varian A-60 spectrometer with tetramethylsilane as an internal standard. IR spectra of **6** were made on a KBr dish using a Perkin–Elmer, 337, Grating Spectrophotometer.

Chromatography and spray reagents. All chromatography was carried out at room temp. Ascending PC was employed using Whatman 3 MM paper and *n*-BuOH–HoAc–H_2O (4:1:5). TLC on polyamide and silica gel layers were also used with MeOH–H_2O (9:1) and MeOH–$CHCl_3$ (1:99). Colour sprays used were 3% aq. $FeCl_3$ and 0·1 M $Na_2MoO_4.2H_2O$.[16] Fluorescent colours of the various thin-layer zones were also noted under UV 254 nm light.

Extraction of bromophenols from sea water of the "Polysiphonia Brodiaei-*Zone*". Six l. of sea water were collected from this algal zone on the rocky shore near the Kristineberg Zoological Station. After filtration through Munktell paper (A3-90-700) the volume was reduced to 1 l. by rotary evaporation, made to 0·05 N HCl and heated to 70° for 0·5 hr to hydrolyze any ester sulphates. The sea water was subsequently shaken repeatedly with EtoAc. The EtoAc fractions were combined and cooled to −10° and ice removed by filtration. The EtoAc extract (approx. 600 ml) was reduced in volume to about 5 ml, placed in a reactivial and freeze-dried. Subsequent analysis was carried out by GLC–MS.

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