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BROMOCHLOROPHENOLS AND A BROMINATED DIPHENYLMETHANE IN RED ALGAE

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Key Word Index—*Polysiphonia nigrescens*; *Rhodomela confervoides*; Rhodomelaceae; red algae; bromochlorophenols; brominated diphenylmethanes; GC–MS.

Abstract—Identification of chlorinated bromophenols and a 2,3,2′,3′-tetrabromo-4,5,4′,5′-tetrahydroxydiphenylmethane in *Polysiphonia nigrescens* and *Rhodomela confervoides* was made by stepwise extraction followed by GC–MS analysis. Four different forms of the brominated and brominated-chlorinated benzyl alcohols in red algae are suggested (i) free phenols, (ii) sulphated potassium salts, (iii) constituents of brominated diphenylmethanes and (iv) part of the red pigment floridorubin.

INTRODUCTION

A number of simple brominated phenols have been found in red seaweeds of the family Rhodomelaceae, where especially *Rhodomela* and *Polysiphonia* species have been investigated [1, 2]. Hodgkin *et al.* [3] described a 2,3-dibromobenzyl alchohol 4,5-disulphate, dipotassium salt from *Polysiphonia lanosa*. Glombitza and Stoffelen [4] corrected the structure to 2,3-dibromo-5-hydroxy-1′,4-disulphate, dipotassium salt. In 1975 Weinstein *et al.* [5], who reinvestigated *Rhodomela larix*, stated that 2,3-dibromo-5-hydroxybenzyl-1′,4-disulphate, dipotassium salt is the only brominated compound present in the alga and that simple bromophenols reported in algae are probably artifacts of the isolation procedure. In 1976 Chevolot-Magueur *et al.* [6] reported the presence of a new brominated compound 5,6,3′,5′-tetrabromo-3,4,2′,4′,6′-pentahydroxydiphenylmethane isolated from the ethanolic extract of *Rytiphlea tinctoria* after treatment with diazomethane. Lundgren and Theander [7] also isolated and identified a 2,3,2′,3′-tetrabromo-4,5,4′,5′-tetrahydroxydiphenylmethane from *P. brodiaei*.

In the present investigation some new chlorinated bromophenols and the same diphenylmethane as in *P. brodiaei* have been identified in *P. nigrescens* and *Rhodomela confervoides*. Great care has been taken to extract the algal material in the mildest possible way to avoid the formation of artifacts due to either enzymatic or chemical reaction during the isolation procedure.

RESULTS AND DISCUSSION

The analyses of *Rhodomela confervoides* were made in many different ways. EtOAc I gave 5 different bromo- and bromochlorophenols (Tables 1 and 2), all of which are 4,5-dihydroxybenzyl alcohols. The aldehydes found in

Table 2. Bromo- and bromochloro compounds obtained by different extraction procedures in *Rhodomela confervoides*

Extraction procedure	MW(TMSi) of compounds
(A) EtOAc I	434, 468, 512 and 590
EtOAc II	434, 512, 546 and 590
EtOAc III	424, 434, 468, 512, 546, and 590
ppt	512 and 590
(B) 1 H_2O pH 5	434, 468, 512, 546 and 590
2 80% Me_2CO pH 5	434, 512 and 590
3 80% Me_2CO in buffer pH 8 9	434, 512 and 590
(C) 1 H_2O pH 5	434, 512 and 590
2 80% Me_2CO pH 5	434, 512 and 590
(D) 1 Buffer pH 8 9 Algal material deep frozen Sonicated 5 min	434, 512, 546, 590 and 832
2 80% Me_2CO in buffer pH 8 9 Algal material deep frozen Sonicated 5 min	434, 512, 546 and 590
3 80% Me_2CO in buffer pH 8 9 Algal material deep frozen Sonicated 45 min	434, 512, 546 and 590
4 80% Me_2CO in buffer pH 8 9 Algal material fresh. Sonicated 5 min	434, 512, 546 and 590

Table 1. Bromo- and bromochloro compounds from *Rhodomela confervoides* and *Polysiphonia nigrescens*

MW (TMSi)	No. of Br or Cl atoms	No. of TMSi groups	GLC R_t at 100–230° at 5°/min	Terminal group
424	2 Br	2	12.2	CH_2OH
434	1 Br	3	16.1	CH_2OH
468	1 Br, 1 Cl*	3	21.5	CH_2OH
512	2 Br	3	22.3	CH_2OH
546	2 Br, 1Cl*	3	23.0	CH_2OH
590	3 Br	3	25.2	CH_2OH
832	4 Br	4	71.2	

* Postions of the Br and Cl atoms have not been determined

this investigation are not reported, as they are considered to be artifacts [5, 8]. A precipitate was obtained when the aqueous solution was made alkaline. On GC–MS analysis this consisted of 2,3-dibromo- and 2,3,6-tribromo-4,5-dihydroxybenzyl alcohol. Extraction of the alkaline H_2O phase gave the same compounds as in ETOAc I except for the monobromo-monochlorophenol, which was absent.

Ethyl acetate extract III contained an additional bromophenol, 3,5-dibromo-*p*-hydroxybenzyl alcohol, and the monobromomonochlorophenol was again present. Acid hydrolysis removes sulphate groups [3]or decomposes floridorubin so that the bromophenols of floridorubin can be identified [9]. Floridorubin [10] and the diphenylmethanes are extracted by EtOAc from an acid H_2O solution, and are therefore removed from H_2O solution by EtOAc I. The presence of bromochlorophenols both before and after acid hydrolysis indicates that they are esterified with sulphate like the bromophenols. Bromochlorophenols have so far only been identified in floridorubin isolated from the red alga *Lenormandia prolifera* [9].

In B (Table 2) algal material was extracted with H_2O, 80% Me_2CO and 80% Me_2CO in buffer. The bromochlorophenols were only present in the first H_2O extract but 2,3-dibromo- and 2,3,6-tribromo-4,5-dihydroxybenzyl occurred in all 3 extracts. The quantities of bromochlorophenols are obviously much smaller than the quantities of bromophenols in the algae.

In C (Table 2) the H_2O phase was heated before extraction to ensure that all sulphated phenols or floridorubin were hydrolysed. In these EtOAc–extracts no bromochlorophenols were detected, possibly because the bromochlorophenols are masked by the very large amounts of bromophenols.

In A, B:1 and D only bromophenols, which are not esterified with sulphate, are extracted with EtOAc and the GLC peaks from bromochlorophenols are well separated bromophenols and thus could be analysed by GC–MS (see Table 1). This also explains why the bromochlorophenols of the red algae *P. nigrescens* and *Rhodomela confervoides* have not been discovered before, in spite of the many investigations made on these algae [1, 2, 5].

In D (Table 2) the algal material was extracted with buffer or 80% Me_2CO in buffer. There was no difference in the content of phenols when fresh or deep frozen algal material were extracted, when buffer or 80% Me_2CO in buffer was used or when the algal material was sonicated for 5 or 45 min. In D:1 a 2,3,2′,3′-tetrabromo-4,5,4′,5′-tetrahydroxydiphenylmethane was discovered with a long R_t (Table 1). D:2, D:3 and D:4 were not analysed for the diphenylmethane

Four other red algae (Table 3) were extracted with H_2O *P. nigrescens* was the only alga that contained the monobromo monochlorophenol and the 2,3,2′,3′-tetrabromo-4,5,4′,5′-tetrahydroxydiphenylmethane in EtOAc I. *P. elongata* contained only 2,3-dibromo- and 2,3,6-tribromo-4,5-dihydroxybenzyl alcohol. *Odonthalia dentata* and *Bronghiartella byssoides* contained lanosol and 3,5-dibromo-*p*-hydroxybenzy alcohol in both EtOAc I and II.

A major difference is seen between on one hand the two red algae *P. nigrescens* and *Rhodomela confervoides*, which contain bromochlorophenols and the brominated diphenylmethane and on the other 3 red algae *P. elongata*, *Brongniartella byssoides* and *Odonthalia dentata* which lack these compounds. All identifications of the phenols and the tetrabromodiphenylmethane were made from the MS of the TMSi derivatives after GC–MS. The MWs of the TMSi derivatives and their other characteristics are given in Table 1.

The fragmentation pattern of all the benzyl alcohols are very similar. The MS of the compounds have been compared with those of authentic compounds and those of compounds isolated and reported from other algae [9, 11]. Acid hydrolysis of authentic tetrabromotetrahydroxydiphenylmethane did not produce any bromophenols. The bromophenols identified in floridorubin after acid hydrolysis [9] may be bound by other links than the carbon–carbon links of tetrabromotetrahydroxydiphenylmethane. In red algae bromine has been identified in chloroplasts of *Lenormandia prolifera* by X-ray microanalyses [12].

Glombitza *et al.* [13] were the first to publish a comparison of free and esterified lanosol in red algae. The results of the present paper point to the possibility that the bromophenols and the bromochlorophenols may occur in at least 4 different forms in the red algae: (i) as simple phenols, (ii) as sulphated potassium salts, (iii) as brominated diphenylmethanes and (iv) as part of floridorubin. The algae of this investigation that contain both bromochlorophenols and tetrabromodiphenylmethanes are also reported to contain floridorubin. Whether this is valid for all red algae containing bromophenols remains to be seen. Until now the bromochlorophenols have been overlooked, but stepwise extraction of bromophenols from the aqueous solution of algal will reveal their presence.

EXPERIMENTAL

Rhodomela confervoides (Huds.) Lamour., *Polysiphonia nigrescens* (Huds.) Grev., *Odonthalia dentata* (L.) Lyngb., *Polysiphonia elongata* Harv. and *Brongniartella byssoides* (Good et Wood) Schmitz were collected in August and September 1976 in the vicinity of Kristineberg's Biological Station on the Swedish West Coast and immediately deep frozen except for some algal material which was kept in seawater at 10° in daylight for some days before extraction. When not otherwise stated the algae were treated as follows: Samples of 0.5–1 g fr. wt of the deep frozen alga were ground with liquid N_2 to a powder. The powder was immediately sonicated for 5 min with 100 ml solvent. The solvent was either H_2O, buffer (0.2 M glycine–NaOH pH 8.9), 80% Me_2CO or 80% Me_2CO in 0.2 M glycine–NaOH buffer pH 8.9. After filtration and evaporation of Me_2CO if present, the aq. soln was partitioned × 3 with 50 ml of EtOAc.

Table 3. Bromo- and bromochloro compounds found in red algae

	Polysiphonia nigrescens	*Polysiphonia elongata*	*Odonthalia dentata*	*Brongniartella byssoides*
EtOAc I MW (TMSi)	468, 512 and 832	512 and 590	424 and 512	424 and 512
EtOAc II MW (TMSi)	424 and 512	512 and 590	424 and 512	424 and 512

The pooled EtOAc fractions were evapd and the residue redissolved in a small vol. of EtOAc, transferred to a micro-silylation vessel and concd to dryness under N_2 and over P_2O_5. BSTFA (*N,O-bis*-trimethylsilyltrifluoroacetamide) and MeCN were added (20 ml each), the mixture heated at 60° for 15 min and subjected to GC–MS. Acid hydrolysis was achieved by heating the aq. soln at pH 5 for 10 min. On cooling, the resulting soln was subjected to the same extraction and silylation procedure as above. The ppt. was dried over P_2O_5 before silylation. Acid hydrolysis of 2,3,2',3'-tetrabromo-4,5,4',5'-tetrahydroxydiphenylmethane was performed by heating an aq. soln of the compound at pH 1 for 15 min. After cooling, the resulting soln was subjected to the same extraction and silylation procedure as before. Analyses of the TMSi derivatives were made by GC–MS [14] using a 1.22 m × 4 mm silanized glass column packed with 3% SE 30 on Chromosorb W. The pre-column was packed with the same material. He was used as carrier at a flow rate of 25 ml/min; the precolumn was kept at 230°. 2,3,2',3'-Tetrabromo-4,5,4',5'-tetrahydroxydiphenylmethane was synthesized by Dr Lennart Lundgren, Dept. of Chemistry II, Agricultural College of Sweden. The algal material was treated in the following way; *Rhodomela confervoides*: (A) The algal material was sonicated in H_2O (pH 5). After EtOAc extraction (EtOAc I) the remaining H_2O phase was made alkaline (pH 8) with 0.1 M NaOH. The ppt. was filtered and dried for GC–MS analysis. The H_2O phase was then extracted with EtOAc (EtOAc II). Remaining H_2O phase was acidified to pH 2 with 0.1 M HCl and heated for 10 min. After cooling the H_2O phase was extracted with EtOAc (EtOAc III). (B) The algal material was extracted first with H_2O, then with 80% Me_2CO and lastly ×3 with 80% Me_2CO in buffer. (C) the algal material was divided into two: one part was extracted with H_2O and the other with 80% Me_2CO. After filtering the algal material and evaporating Me_2CO if present, the ester sulphates were removed by heating the aq. solns for 10 min (pH 5) After cooling, the solns were extracted with EtOAc. (D) The algal material was divided into 4, 3 of which were deep frozen. D:1 was extracted at pH 8.9 in buffer. D:2 was extracted at pH 8.9 in 80% Me_2CO in buffer. D:3 was sonicated for 45 min instead of usual 5 min, otherwise treated as D:2. In D:4 fresh algal material was used instead of deep frozen, otherwise extracted as D:2. *Polysiphonia nigrescens, P. elongata, Odonthalia dentata and Bronghiartella byssoides*: the algal material was extracted with H_2O. The remaining aq. soln (pH 5) after the first EtOAc-extraction (EtOAc I) was heated for 10 min. After cooling, the soln was again partitioned with EtOAc (EtOAc II, Table 3).

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