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BROMO-COMPOUNDS OF THE RED ALGA *LENORMANDIA PROLIFERA*

PETER SAENGER*, MARIANNE PEDERSÉN† and KINGSLEY S. ROWAN‡

*Sciences Section, The Southern Electric Authority of Queensland, P.O. Box 403, Brisbane, Australia;
†Institute of Physiological Botany, University of Uppsala, Box 540, S-751 21 Uppsala, Sweden;
‡School of Botany, University of Melbourne, Parkville, Australia

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Abstract—Six bromo-compounds and one bromo–chloro-compound have been detected in *Lenormandia prolifera* (C.Ag.) J. Agardh (Amansieae; Rhodomelaceae). Hydrolysis of the red pigment floridorubin from the same alga yielded five bromo-, one bromo-chloro and one chloro-phenol. The two main phenols of floridorubin were 2,3-dibromo-4,5-dihydroxy benzyl alcohol (lanosol) and 3,5-dibromo-*p*-hydroxybenzyl alcohol.

INTRODUCTION

Members of the Amansieae are known to be particularly rich in Br with concentrations up to 2.5% of the dry wt [1]. Much of this Br is contained in organic compounds including bromophenols [2–9]. Péguy [5] has suggested that in *Halopithys pinastoides* (Gmel.) Kuetzing, the bromophenolic complex consists of a number of closely related substances which are more or less stable but which in part are subject to natural hydrolysis.

We have employed a GC–MS technique [11] to study the bromophenolic complex of *Lenormandia prolifera* (C.Ag.) J. Agardh (Amansieae; Rhodomelaceae) and have also examined the brominated red pigment floridorubin using a similar technique. The preliminary findings regarding its molecular composition are presented and discussed in this paper.

RESULTS AND DISCUSSIONS

Bromo-compounds

Twelve Br-compounds were found in the dried material of *L. prolifera*; the MWs of the TMSi derivatives and their other characteristics are given in Table 1. The Et ether is an artifact and no monohalogenated compounds were observed. Of the three tri-Br phenols identified by MS, compounds **1** and **2** have been previously reported [10,13].

Table 1. Bromo-compounds from *Lenormandia prolifera*

MW (Silylated)	No. of Br or Cl groups	No. of TMSi groups	GLC R_t at 100° at 6°/min	Proposed terminal group
262 (**4**)	2Br	0	11.7	CHO
336 (**5**)	2Br	1	15.1	CH_2OH
350 (**6**)	2Br	1	15.9	CHO
366 (**8**)	1Br, 1Cl	2	16.3	—
424*	2Br	2	18.3	CH_2OH
428* (**2**)	3Br	1	18.7	CHO
438*	2Br	2	20.4	CHO
468*	2Br	2	21.2	CH_2OEt
502* (**3**)	3Br	2	21.0	CH_2OH
512*	2Br	3	21.8	CH_2OH
540 (**7**)	2Br	3	23.0	—
590* (**1**)	3Br	3	24.2	CH_2OH

* Configuration given [9, 10, 12].

Three diBr-compounds (**4–6**) did not exhibit the loss of $(M\text{-}88)^+$ or $(M\text{-}103)^+$, characteristic of *ortho*-dihydroxy substituents. Compound **5** showed the loss of CH_2O– from the ion $(M\text{-}Me)^+$. In these compounds the position of the Br atoms has not been determined. Compound **7** (M^+ 540) with 2 Br atoms has not been previously reported. The terminal group of **7** is not known, although according to the MS fragmentation pattern it might be a CH_2COOH– group. Compounds of the formula $C_6(SO_3K)_2Br(OH)_2COOH$[3] and $C_6(SO_3K)_2Br_2OHCOOH$[2] have been reported from *Halopithys pinastroides* (Gmel.) Kuetz. and *Vidalia volubilis* (L.) J. Ag. respectively.

Floridorubin

When floridorubin was analysed by direct inlet MS, two compounds were observed, at 180° and 200° respectively. The first of these contains three Br atoms and has a M^+ 342. The second compound contains one Cl atom and two Br atoms and has a M^+ 312.

GLC of the TMSi derivative of floridorubin did not reveal any peaks until acid hydrolysis, after which the TMSi derivatives of ten phenols were observed (Table 2). Three of the phenols (**9**, **10**, **11**) were chlorinated in addition to having Br substituents. According to the MS fragmentation pattern compound **11** corresponds to lanosol with a Br atom replaced by a Cl atom. The most abundant phenols were 2,3-dibromo-4,5-dihydroxybenzyl alcohol (lanosol) and 3,5-dibromo-p-hydroxybenzyl alcohol. Only alcoholic phenols were observed in the hydrolysis products of floridorubin from *L. prolifera*. The three Et ethers are artifacts [14]. In the analysis of the bromophenols of this alga, both aldehyde and alcohol groups were observed. Experiments with pure lanosol revealed that after standing or after boiling the alcohol, aldehydes may also be artifacts produced during the analytical procedure [9,15]. Elemental analysis of floridoru-

Table 2. Hydrolysis products of floridorubin obtained by ethyl acetate extraction

MW (Silylated)	No. of Br or Cl groups	No. of TMSi groups	GLC R_t at 100° at 6°/min	Proposed terminal group
302 (**9**)	1Cl	2	19.7	CH_2OH
336	2Br	1	13.0	CH_2OH
346*	1Br	2	14.2	CH_2OH
380	2Br	1	16.1	CH_2OEt
424 (**10**)	1Cl, 1Br	2	21.0	CH_2OEt
424*	2Br	2	18.4	CH_2OH
434*	1Br	3	21.1	CH_2OH
468 (**11**)	1Cl, 1Br	3	20.2	CH_2OH
468*	2Br	2	20.7	CH_2OEt
512*	2Br	3	22.3	CH_2OH

* Configuration given [9, 10, 12].

bin gave the following: C: 17.05%; H: 1.13%; O: 26.0%; Br: 23.5%; Cl: 4.7% and N: 1.2%. Both the amino acid and nitrogen base analyses of floridorubin yielded negative results.

Br- and Cl-substituted phenols have sofar only been identified from *L. prolifera* although monoterpenes containing both halogens have been reported from the Rhodophyta [7]. We have no evidence about chlorinated bromophenols in floridorubin from other red algae. Nevertheless the same results relating to chlorinated bromophenols in *L. prolifera* have been found in samples of floridorubin prepared on two separate occasions.

EXPERIMENTAL

L. prolifera was collected from the shore-platform at Point Lonsdale, Victoria, Australia and subsequently air-dried for 4 days and oven-dried at 60° for 2 hr. After drying, the algal material was pulverised.

Preparation of bromophenols. Pulverised algal material (1.9 g) was treated consecutively with 200, 100 and 100 ml of boiling 80% Me_2CO for 1 hr and the total extract was pooled. The Me_2CO was removed by rotary evaporation after filtration. To the aq soln, HCl was added to give a final concn of 0.05 N HCl, and the mixture was boiled at 100° for 15 min. On cooling, the mixture was filtered through Celite, extracted ×3 with 30 ml of EtOAc. The EtOAc was dried and evaporated and the residue taken up in 3 ml × 3 washings of hot H_2O and centrifuged for 10 min. The supernatant was then freeze-dried and prepared for GLC.

Preparation of floridorubin. Fresh algal material was extracted with H_2O and after the filtration, the vol was reduced by rotary evaporation. The concentrated soln was purified by passing through a 200 ml Sephadex G 10 column and the fractions occurring near the eluant vol of 150–250 ml were collected. The fractions were dried over H_2SO_4 *in vacuo*.

Various hydrolysis products were prepared. For GLC 2 mg of floridorubin was dissolved in H_2O (10 ml) and acidified to give a final concn of 0.05 N HCl. The soln was boiled at 100° for 10 min, extracted with EtOAc and the EtOAc was subsequently evaporated under a stream of N_2 and dried over P_2O_5. For nitrogen base analyses, the method of Markham as given by Rowan [12] was used. 3 drops of the floridorubin soln were evaporated to dryness and *three capillary cm* of perchloric acid added. The tube was sealed and heated at 100° for 1 hr. The resultant soln was applied to Whatman No. 3 paper with an adenine marker on a control strip. A descending PC was run with *iso*PrOH–HCl–H_2O (85:22:18). After 7 hr, chromatograms were dried and examined under UV light. For amino acid hydrolysis, 3 ml floridorubin soln were added to 0.5 ml of 14 N HCl and boiled under reflux for 2 hr at 100°. The soln was subsequently evaporated under red pres and 0.5 ml of 80% MeOH–H_2O added to the residue. A control was set up by evaporating 0.7 ml of floridorubin soln and adding 0.1 ml of 80% MeOH–H_2O. A PC was run on Whatman No. 3 paper using *n*-BuOH–HOAc–H_2O (4:1:1) as solvent. After drying it was sprayed with ninhydrin in *n*-BuOH, and heated at 100° for 15 min prior to examination.

GLC. Dried samples of bromophenols and floridorubin hydrolysis products were silylated by adding 20 μl of MeCN and 20 μl of BSTFA (*N,O-bis*-TMSi-trifluoroacetamide) containing 1% TMCS as catalyst. The reactive vials were kept at 40° for 15 min prior to use. Analysis of the TMSi derivatives carried out on a FID instrument using a 1.2 m × 4 mm, silanized glass column packed with 3% SE 30 on a 80–100 mesh chromosorb W. A carrier gas (97% N_2 and 3% H_2) flow rate of 50 ml/min was maintained. Injector and detector temp. were maintained at 220° and 240° respectively.

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