Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 62 (2003) 1221–1224
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Antibacterial bromophenols from the marine red alga *Rhodomela confervoides*

Nianjun Xu[a,b,*], Xiao Fan[a], Xiaojun Yan[b], Xiancui Li[a], Rongli Niu[a], C.K. Tseng[a]

[a]*Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, PR China*
[b]*Key Laboratory of Marine Biotechnology, Ningbo University, Ningbo 315211, PR China*

Received 14 November 2002

Abstract

Two bromophenols, together with three known compounds, were isolated from the methanolic extract of the marine alga, *Rhodomela confervoides*. By means of MS and NMR spectroscopic analyses, they were identified as 3-bromo-4-[2,3-dibromo-4,5-dihydroxyphenyl] methyl-5-(hydroxymethyl) 1,2-benzenediol (**1**) and 3-bromo-4-[2,3-dibromo-4,5-dihydroxyphenyl] methyl-5- (ethoxymethyl) 1,2-benzenediol (**2**). Three known compounds were also isolated, namely 3-bromo-4-[2,3-dibromo-4,5-dihydroxyphenyl] methyl-5-(methoxymethyl) 1,2-benzenediol (**3**), 4,4′- methylenebis [5,6-dibromo-1,2-benzenediol] (**4**) and bis (2,3-dibromo-4,5-dihydroxybenzyl) ether (**5**). Compound **5** was the most active against five strains of bacteria with the MIC less than 70 μg/ml, while compounds **2**, **3** and **4** exhibited moderate activity.

Keywords: *Rhodomela confervoides*; Rhodomelaceae; Red alga; Bromophenol; Antibacterial activity

1. Introduction

Marine algae serve as important resources for bioactive natural products (Iliopoulou et al., 2002; Metzger et al., 2002). Many of these compounds show antibacterial activities (Vairappan et al., 2001; Vlachos et al., 1999), which prompted us to investigate the Chinese algae. In our previous studies, we screened the ethanol extracts of some widely distributed algae in China, and found five species showing prominent antibacterial activities (Xu et al., 2002). *Rhodomela confervoides*, a marine red alga belonging to the family of Rhodomelaceae, showed the most remarkable activities, and was thus selected as the target material to isolate potential antibiotics. A number of bromophenol metabolites have been previously isolated from the genus *Rhodomela* (Kurata and Amiya, 1980; Katsui et al., 1967; Pedersen et al., 1974; Pederson, 1978; Phillips and Towers, 1981; Weinstein et al., 1975; Minoru et al., 1980), some of which have been shown to significant feeding-deterrent activities (Kurata et al., 1997) and α-glucosidase inhibition activities (Kurihara et al., 1999). In this antibacterial bioassay guided isolation study, we obtained two novel bromophenols, 3-bromo-4-[2,3-dibromo-4,5-dihydroxyphenyl] methyl-5- (hydroxymethyl) 1,2-benzenediol (**1**) and 3-bromo-4- [2,3-dibromo-4,5-dihydroxyphenyl] methyl-5-(ethoxymethyl) 1,2-benzenediol (**2**), together with three known compounds 3-bromo-4-[2,3-dibromo-4,5-dihydroxyphenyl] methyl-5-(methoxymethyl) 1,2-benzenediol (**3**), 4,4′-methylenebis [5,6-dibromo-1,2-benzenediol] (**4**) and bis (2,3-dibromo-4,5-dihydroxybenzyl)ether (**5**).

2. Results and discussion

The HREI MS of compound **1** showed a [M] at *m*/*z* 495.8134, corresponding to the molecular formula $C_{14}H_{11}^{79}Br_3O_5$ (calc. 495.8157). The mass spectrum of **1** also gave the tribrominated molecular ion peaks cluster at *m*/*z* 502, 500, 498, 496 with a ratio of 1:3:3:1. The IR spectrum (KBr) showed two absorption bands for hydroxyl groups at 3477 and 3425 cm^{-1} and characteristic absorption bands for aromatic rings at 1608, 1577, 1491, 1469 cm^{-1}. The ^1H NMR spectrum of **1** in acetone-d_6

* Corresponding author. Tel.: +86-574-8760-0458; fax: +86-574-8760-0590.
E-mail address: njxu1973@hotmail.com (N. Xu).

doi:10.1016/S0031-9422(03)00004-9

exhibited two singlets attributed to methylene protons at δ 4.12(2H, *s*, H-7) and δ 4.42(2H, *s*, H-8), and two singlets assigned to aromatic protons at δ 6.08(1H, *s*, H-6′) and 7.09 (1H, *s*, H-6). The ^{13}C NMR and DEPT spectra of **1** displayed 14 carbon signals attributed to two *penta*-substituted benzene rings ($\delta > 142$ ppm) and two methylenes (δ 38.6*t* and δ 62.1*t*) (Table 1). All of the above spectral data proved that **1** possessed a tribrominated diarylmethane structure with substituent groups of four hydroxyls and one hydroxymethyl, respectively. In the HMBC spectrum, cross-peaks from phenolic protons to their correlated long range carbons unambiguously established the substituted patterns of the aromatic rings. Long range correlations from H_2-7 to C-2′, C-6′, C-3 and C-5 confirmed the diarylmethane structure of 1, and correlations from H_2-8 to C-4, C-6, and from H-6′ to C-2′, C-4′, C-5′, C-7, and from H-6 to C-2, C-4, C-3, C-8 revealed that the substitution groups were 2′,3′-dibromo-4,5-dihydroxy and 3′-bromo-1,2-dihydroxy respectively. Accordingly, **1** was determined to be 3-bromo-4-[2,3-dibromo-4,5-dihydroxyphenyl] methyl-5-(hydroxymethyl) 1,2-benzenediol.

The HREI MS of compound **2** showed the $[M]^+$ at *m*/*z* 523.8466, in agreement with the molecular formula $C_{16}H_{15}^{79}Br_3O_5$ (calc. 523.8470). The EI MS spectrum of **2** gave a tribrominated molecular ion peak cluster at *m*/*z* 530, 528, 526 and 524 in a ratio of 1:3:3:1. The IR and NMR spectra of **2** were similar to those of compound **1**, the IR spectrum (KB) showed absorption bands for hydroxyl groups at 3527 and 3415 cm^{-1} and strong absorption for aromatic rings at 1568, 1487, 1469 cm^{-1}. Analysis of the ^{1}H NMR spectrum of **2** indicated the presence of one ethyloxyl groups at δ1.06 (3H, *t*, $J = 6.90$,–OCH_2CH_3) and δ 4.25 (2H, *s*,–OCH_2CH_3), two methylene protons at δ 4.13(2H, *s*, H-7) and δ 3.40 (2H, q, $J = 7.2$, H-8), and two aromatic protons at δ 6.08 (1H, *s*, H-6′) and 7.00 (1H, *s*, H-6). The ^{13}C NMR and DEPT spectra of **2** displayed 16 carbon signals attributed to two *penta*-substituted benzene rings and one methyl group (δ 14.7*q*), three methylenes (δ 38.8*t*, δ 65.5*t* and δ 70.7*t*) (Table 1). Long range ^{1}H/^{13}C correlations observed in the HMBC experiment unambiguously showed the substituted patterns of the aromatic rings was the same as those of compound **1**, while the substituted group in C-8 was an ethyloxyl group. On the basis of these spectroscopic studies, it was concluded that **2** was 3-bromo-4-[2,3-dibromo-4,5-dihydroxyphenyl] methyl-5-(ethoxymethyl)-1,2-benzenediol.

Compounds **3**, **4**, **5** were isolated from the *R. confervoides* for the first time, and had previously been reported from other natural sources (Kazuya and Takashi, 1977; Lundgren et al., 1979; Kurihara et al., 1999). The structures were determined by comparison of their spectral data with the literature values (Fig. 1).

The antibacterial activities of the five isolated bromophenol metabolites were tested against eight strains of Gram positive and Gram negative bacteria by a pour plate method (Negi et al., 1999). The minimum inhibitory concentration (MIC) of the compounds against bacteria is listed in Table 2. Compound **5** showed the most potent inhibitory activity against seven out of eight strains of bacteria, tested with MIC less than 70 μg/ml being effective for five strains whereas compound **3** could inhibit only four bacterial strains at the same MIC. Compounds **2** and **4** exhibited weak antibacterial activity with most of the MIC at 140 μg/ml.

Table 1
NMR spectroscopy data of compounds **1** and **2**[a]

1			**2**		
No.	δ_H	δ_C	No.	δ_H	δ_C
1		131.7*s*			142.8*s*
2		133.5*s*			144.1*s*
3		116.0*s*			114.3*s*
4		142.3*s*			128.8*s*
5		144.4*s*			130.5*s*
6	7.09 (1H, *s*)	115.7*d*		7.00 (1H, *s*)	115.6*d*
7	4.12 (2H, *s*)	38.6*t*		4.13(2H, *s*)	38.8*t*
8	4.42 (2H, *s*)	62.1*t*		4.25 (2H, *s*)	70.7*t*
				3.40 (2H, *q*, $J = 7.2$)	65.5*t*
				1.06 (3H, *t*, $J = 6.90$)	14.7*q*
1′		127.7s	1′		131.8*s*
2′		114.1s	2′		115.6*s*
3′		113.1s	3′		112.9*s*
4′		142.9s	4′		142.7*s*
5′		144.8s	5′		144.7*s*
6′	6.08 (1H,s)	114.2d	6′	6.08 (1H, *s*)	114.3*d*

[a] NMR data were measured in acetone-d_6 at 300 MHz for proton and at 75 MHz for carbon. The assignments were based on DEPT, ^{1}H–^{1}H COSY, HMQC and HMBC experiments.

3. Experimental

3.1. General

Melting points were determined on an XT-4 micro melting point apparatus and were uncorrected. Optical rotations were measured on a Rudolph Research Autopol

Table 2
Antibacterial activities of the compounds against eight strains of bacteria[a]

Test bacteria	Compounds				
	(1)	**(2)**	**(3)**	**(4)**	**(5)**
Staphylcoccus aureus ATCC29213	+	++	++	+	++
Staphylcoccus aureus 02-60	+	+	++	+	++
Staphylcoccus epidermidis ATCC12228	+	+	++	+	+++
Staphylcoccus epidermidis 02-4	–	+	++	+	+
Escherichia coli ATCC25922	–	+	–	+	+
Escherichia coli 02-26	–	–	–	–	–
Pseudomonas aeruginosa ATCC27853	–	–	–	+	++
Pseudomonas aeruginosa 02-29	–	–	–	+	++

[a] MIC; +++: 35 μg/ml, ++: 70 μg/ml, +: 140 μg/ml,–: >140 μg/ml.

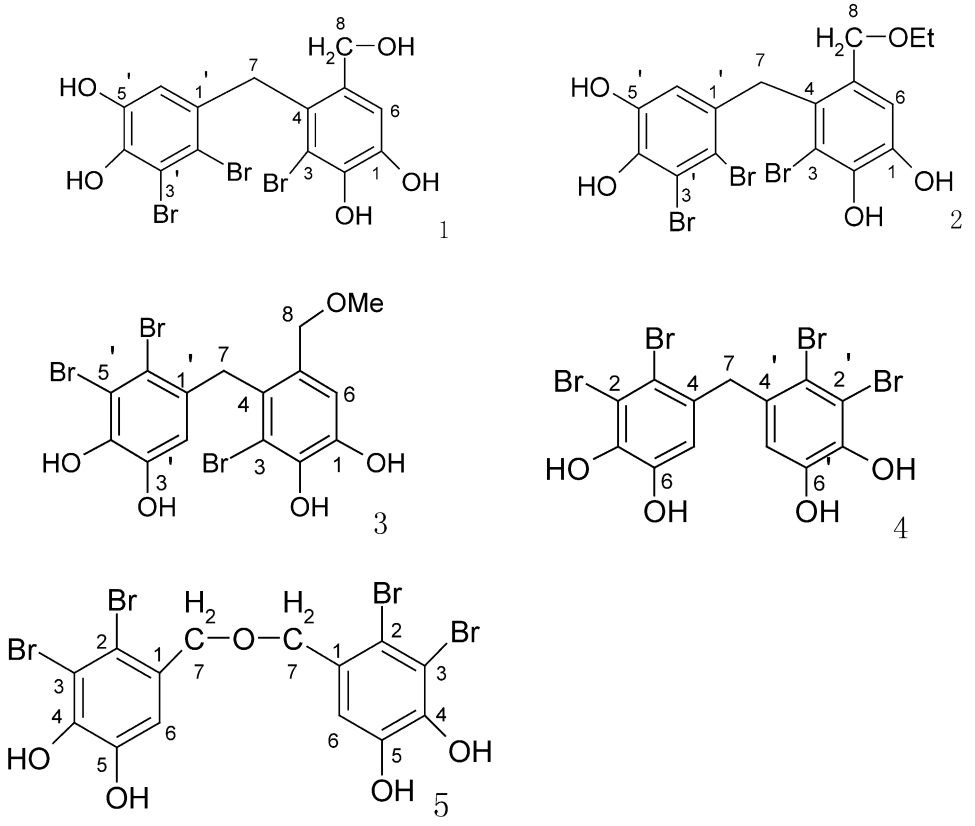

Fig. 1. The chemical formulae of compounds **1**–**5**.

III automatic polarimeter. IR spectra were recorded as KBr disks on a Nicolet Impact 400 FT-IR Spectrophotometer. 1D- and 2D-NMR spectra were obtained at 300 and 75 MHz for ^{1}H and ^{13}C, respectively, on an Inova 300 MHz spectrometer in acetone-d_6 with solvent peaks as references. EIMS and HREIMS data were measured with a Micromass Autospec-Ultima ETOF spectrometer. CC was performed with silica gel (160–200 mesh), Bio-Beads SX3 (200–400 mesh), RP-18 reversed phase silica gel (43–60 μm) and Sephadex LH-20. TLC was carried out with glass precoated silica gel GF_{254} plates. Spots were visualized under UV light or by spraying with 5% H_2SO_4 in 95% EtOH followed by heating. HPLC was performed using an Alltima C18 10 μ preparative column (22×250 mm).

3.2. Plant material

The alga *R. confervoides* was collected at the coast of Qingdao, China in May 2001, and was identified by Professor B.M. Xia. The voucher sample was deposited at the Department of Seaweed Chemistry, Institute of Oceanology, Chinese Academy of Sciences (voucher number: 2001-002).

3.3. Extraction and isolation

The air-dried alga *R. confervoides* (14.44 kg) was soaked in MeOH–H_2O (95:5) at room temperature for 3×2 days. The methanolic extract of the alga was filtered and evaporated under reduced pressure at $<40\ ^{\circ}C$, and the residue was dissolved in distilled water and then partitioned using EtOAc. The EtOAc extract (594.6 g) was loaded into a silica gel column and then subjected to gradient elution with mixtures of $CHCl_3$–MeOH of increasing polarity. The fraction eluted by $CHCl_3$–MeOH (3:1 and 5:1) was purified by size-exclusion chromatography over Bio-Beads SX-3 with $CHCl_3$–EtOAc (1:1) as eluent. In the $CHCl_3$–MeOH (3:1) fraction, 61.3 mg of **1** and 52.8 mg of **2** were obtained by HPLC purification (methanol:water 4:1). By recrystallization, 1.17 g of **3**, 1.08 g of **4** and 388.7 mg of **5** were obtained in the $CHCl_3$–MeOH 3:1 and 5:1 fraction, respectively.

3.4. 3-Bromo-4-[2,3-dibromo-4,5-dihydroxyphenyl] methyl-5-(hydroxymethyl) 1,2-benzenediol *1*

Yellowish white needles (61.3 mg); m.p. 127–129 °C. IR (KBr): 3477, 3425, 1684, 1608, 1577, 1491, 1469, 1439, 1402, 1277, 1190, 1093, 1016, 949, 858, 810 cm^{-1}; EI MS: *m*/*z* 502, 500, 498, 496, 484, 482, 480, 479, 467, 465, 463, 461, 403, 401, 399, 322, 320, 242, 231, 229, 213, 184, 149, 139, 82, 80; HREI MS:*m*/*z* 495.8134 ($C_{14}H_{11}^{79}Br_3O_5$, calc. 495.8157); ^{1}H NMR (300 MHz, acetone-d_6): δ 4.12 (2H, *s*, H-7), 4.42 (2H, *s*, H-8), 6.08 (1H, *s*, H-6′), 7.09 (1H, *s*, H-6). ^{13}C NMR (75

MHz, acetone-d_6): δ 38.6 (*t*, C-7), 62.1 (*t*, C-8), 113.1 (*s*, C-3′), 114.1 (*s*, C-2′), 114.2 (*d*, C-6′), 115.7 (*d*, C-6), 116.0 (*s*, C-3), 127.7 (*s*, C-1′), 131.7 (*s*, C-1), 133.5 (*s*, C-2), 142.3 (*s*, C-4), 142.9 (*s*, C-4′), 144.4 (*s*, C-5), 144.8 (*s*, C-5′).

3.5. 3-Bromo-4-[2,3-dibromo-4,5-dihydroxyphenyl] methyl-5-(ethoxymethyl) 1,2-benzenediol **2**

Yellowish white needles (52.8 mg); m.p. 197–199 °C; IR (KBr): 3527, 3415, 2978, 2875, 1610, 1585, 1568, 1487, 1469, 1406, 1348, 1302, 1271, 1171, 1097, 1076, 1003, 955, 872, 808 cm^{-1}; EI MS: *m*/*z* 530, 528, 526, 524, 484, 482, 480, 478, 467, 465, 463, 461, 403, 401, 399, 384, 357, 355, 353, 322, 320, 293, 291, 242, 213, 184, 161, 160, 139, 121, 58; HREI MS:*m*/*z* 523.8466 ($C_{16}H_{15}^{79}Br_3O_5$, calc. 523.8470); ^{1}H NMR (300 MHz, acetone-d_6) δ 1.06 (3H, *t*, $J = 6.90$, CH_3-10), 3.40 (2H, *q*, $J = 7.2$, CH_2-9), 4.13 (2H, *s*, H-7), 4.25 (2H, *s*, H-8), 6.08 (1H, *s*, H-6′), 7.00 (1H, *s*, H-6); ^{13}C NMR (75 MHz, acetone-d_6): δ14.7 (*q*, C-10), 38.8 (*t*, C-7), 65.5 (*t*, C-9), 70.7 (*t*, C-8), 114.3 (*d*, C-6′), 115.6 (*s*, C-6), 112.9 (*s*, C-3′), 114.3 (*s*, C-3), 115.6 (d, C-2′), 128.8 (*s*, C-4), 130.5 (*s*, C-5), 131.8 (*s*, C-1′), 142.7 (*s*, C-4′), 142.8 (*s*, C-1), 144.1 (*s*, C-2), 144.7 (*s*, C-5′).

3.6. Antibacterial bioassays

Antibacterial bioassays were carried out using eight strains of bacteria. Four strains,*Staphylcoccus aureus* ATCC29213, *Staphylcoccus epidermidis* ATCC12228, *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853 were ATCC standard bacteria; the other four strains, *S aureus* 02-60,*S epidermidis* 02-04, *E coli* 02-26 and *P aeruginosa* 02-29 were isolated from clinic. Bacteria were maintained at 4 °C in nutrient agar, and strains were successively grown twice in nutrient broth at 37 °C for 24 h to obtain synchronization of cells. It was again sub-cultured in nutrient broth (37 °C for 48 h) for use as inocula. All compounds were diluted suitably in MH broth into various concentrations of 4.4, 8.8, 17.5, 35, 70, 140 μg/ml and were introduced into sterile melted and cooled nutrient agar (20 ml) in separate flasks. Bacterial suspension (100 μl) was added into each flask and the medium was pour-plated aseptically. In the case of the control, only agar and bacterial suspension were used. The growth of each bacterium was observed in terms of colony forming units after incubation at 37 °C for 18 h. The minimum inhibitory concentration was reported as the lowest concentration of the compounds required for complete inhibition of growth of the bacteria being tested. All the assays were carried out in triplicate.

Acknowledgements

The authors are very grateful to Professor Abliz Zeper, Institute of Marina Medica, Chinese Academy of Medical Sciences, for mass spectra measurements. The work was supported by the National Science Foundation (Grant No. 29932030) and national "863" program (Grant No. 2001AA620403).

References

Iliopoulou, D., Vagias, C., Harvala, C., Roussis, V., 2002. C_{15} acetogenins from the red alga *Laurencia obtusa*. Phytochemistry 59 (1), 111–116.

Katsui, N., Suzuki, Y., Kitamura, S., Irie, T., 1967. 5′6-Dibromoprotocatechauldehyde and 2′3-dibromo-4,5-dihydroxybenzyl methyl ether from new dibromophenols from *Rhodomela larix*. Tetrahedron 23, 1185–1188.

Kazuya, K., Takashi, A., 1977. Two new bromophoenols from the red alga, *Rhodomela larix*. Chem. Lett. 12, 1435–1438.

Kurata, K., Amiya, T., 1980. A new bromophenol from the red alga *Polysiphonia urceolata*. Bull. Chem. Soc. Jpn. 53, 2020–2022.

Kurata, K., Taniguchii, K., Takashima, K., Hayashi, I., Suzuki, M., 1997. Feeding-deterrent bromophenols from *Odonthalia corymbifera*. Phytochemistry 45 (3), 485–487.

Kurihara, H., Mitani, T., Kawabata, J., Takahashi, K., 1999. Two new bromophenols from the red alga *Odonthalia corymbifera*. J. Nat. Prod. 62, 882–884.

Lundgren, L., Olsson, K., Theander, O., 1979. Synthesis of some bromophenols present in red algae. Acta Chem. Scand. B. 33, 105–108.

Metzger, P., Rager, M.N., Largean, C., 2002. Botryolins A and B, two tetramethylsqualene triethers from the green microalga *Botryoccus braunii*. Phytochemistry 59, 839–843.

Minoru, S., Nobuhiko, K., Etsuro, K., 1980. Bromophenols from the red alga *Rhodomela larix*. Bull. Chem. Soc. Jpn. 53, 2099–2100.

Negi, P.S., Jayaprakasha, G.K., Jagan, M.R.L., Sakariah, K.K., 1999. Antibacterial activity of tumeric oil: a byproduct from curcumin manufacture. J. Agric. Food Chem. 47, 4297–4300.

Pedersen, P., Saenger, P., Fries, L., 1974. Simple brominated phenols in red algae. Phytochemistry 13 (8), 2273–2279.

Pederson, M., 1978. Bromochlorophenols and a brominated diphenylmethane in red algae. Phytochemistry 17, 291–293.

Phillips, D.W., Towers, G.H.N., 1981. Bromophenols of *Rhodomela larix*: chemotaxonomy of morphological forms. Biochem. Syst. Ecol. 9, 1–3.

Vairappan, C.S., Daitoh, M., Suzuki, M., Abe, T., Masuda, M., 2001. Antibacterial halogenated metabolites from the Malaysian Laurencia species. Phytochemistry 58, 291–297.

Vlachos, V., Critchley, A.T., von Holy, A., 1999. Differential antibacterial activity of extracts from selected Southern African macroalgal thalli. Bot. Mar. 42, 165–173.

Weinstein, B., Rold, T.L., Harrell, C.E., Burns, M.W., Waaland, J.R., 1975. Reexamination of the bromophenols in the red alga *Rhodomela Larix*. Phytochemistry 14, 2667–2670.

Xu, N.J., Fan, X., Han, L.J., Lin, W., Tseng, C.K., 2002. Study on the antibacterial activities of the ethanol extracts of seaweed. Oceanol. Limnol. Sin 33, 265–270.