Title:

Two new algal bromophenols from *Odonthalia corymbifera*

Author Names and Affiliations

Md. Reazul Islam, Daisuke Mikami, and Hideyuki Kurihara*

Faculty of Fisheries Sciences, Hokkaido University, Minato, Hakodate, Hokkaido 041-8611, Japan

*Corresponding Author

Tel and Fax: +81 138 40 5561

Email: kuri@fish.hokudai.ac.jp.

Abstract

Two new algal bromophenols, odonthalol and odonthadione, were isolated from the alga *Odonthalia corymbifera*. Odonthalol was determined as a trimer of brominated hydroxylated benzyl (BHB) units. Three units were connected via ether and methylene bridges. Odonthadione was identified as a hybrid compound of the BHB unit and a unique cyclopentenedione. The cyclopentenedione unit has not been reported from natural origin.

Keywords

Bromophenol; antioxidant; tyrosinase; inhibition

Introduction

Algal bromophenols consist of brominated hydroxylated benzyl (BHB) unit. Rhodomelaceae algae contain monomers and dimers of mono- to tribrominated 3,4-dihydroxybenzyl alcohols and alkyl ethers as typical BHB units.[1] Many kinds of the dimers have been reported and constructed with two BHB units via ether and methylene bridges.[2] However, there are few reports on isolation of bromophenol trimers[3-5] and hybrid bromophenols.[6-8] Algal bromophenols have been investigated for various activities such as antioxidant,[6] enzyme inhibitory,[9-16] feeding deterrent,[17] antimicrobial[18,19] and anti-inflammatory[3] activities. In the present report, two new algal bromophenols, a trimer of BHB units named odonthalol (**1**) and a hybrid compound of a BHB unit and a cyclopentenedione moiety named odonthadione (**3**), were isolated from the alga *Odonthalia corymbifera* (S. G. Gmelin) Greville (Rhodomelaceae).

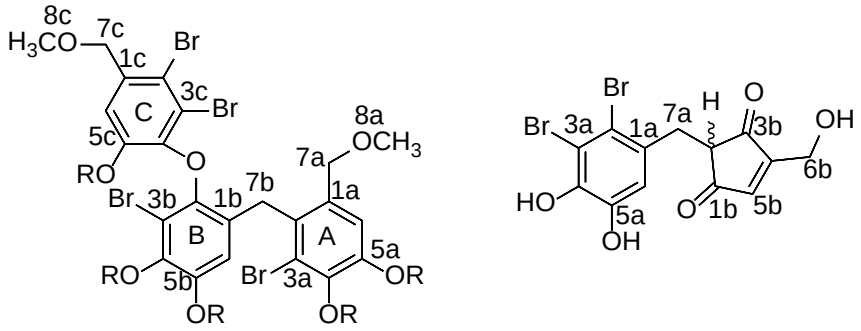


Figure 1. Novel algal bromophenols, Odonthalol (**1**) and odonthadione (**3**).

Results and Discussion

The alga *O. corymbifera* (1.8 kg) was collected at the coast of Hakodate city, Japan (May, 2016) and extracted with 95% MeOH. The EtOAc soluble fraction (19.5 g) of MeOH extract was chromatographed over silica gel, RP-18 column chromatography, and Sephadex LH-20. Final purification was performed by RP-18 HPLC (eluted with 70% aqueous MeOH) to obtain compounds **1**[20] (2.8 mg) and **3**[21] (6.5 mg).

Table 1. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data of odonthalol (**1**) and its permethylated derivative (**2**) in acetone-d_6

	1			**2**			
Position	δ_C, type	δ_H	HMBC	δ_C, type	δ_H	HMBC	ROESY
1a	130.7[a], C			135.7, C			
2a	128.5, C			129.1, C			
3a	115.0[b], C			122.7[f], C			
4a	144.6[c], C			146.9, C			
5a	143.4[c], C			153.0, C			
6a	116.2, CH	6.94, s	2a, 4a, 5a, 7a	113.8, CH	7.11, s	1a, 2a, 4a, 5a, 7a	7a, 8a, 5a-OMe
7a	73.1, CH_2	4.16, brs	ND[g]	73.5, CH_2	4.35, s	1a, 2a, 6a, 8a	6a, 6b
8a	57.8, CH_3	3.14, s	7a	58.2, CH_3	3.22, s	7a	6a, 6b
4a-OMe				60.4, CH_3	3.75, s	4a	
5a-OMe				56.4, CH_3	3.88, s	5a	6a
1b	125.5[a], C			130.5, C			
2b	143.2[d], C			142.6, C			
3b	105.8, C			106.5, C			
4b	142.2[d], C			146.9, C			
5b	143.9, C			152.2, C			
6b	114.1, CH	6.08, s	2b, 4b, 5b	112.8, CH	6.39, s	1b, 2b, 4b, 5b, 7b	7a, 8a, 5b-OMe, 5c-OMe
7b	31.7, CH_2	3.94, brs	ND[g]	32.9, CH_2	4.07, brs	ND[g]	
4b-OMe				60.8, CH_3	3.80, s	4b	
5b-OMe				56.5, CH_3	3.64, s	5b	6b
1c	131.1, C			136.5, C			
2c	117.4, C			116.6, C			
3c	114.0[b], C			122.6[f], C			
4c	145.6[e], C			151.1, C			
5c	145.2[e], C			146.9, C			
6c	113.3, CH	6.78, s	2c, 4c, 5c, 7c	113.7, CH	6.71, s	1c, 2c, 4c, 5c,	6b, 7c, 8c, 5c-

						7c	OMe
7c	75.1, CH_2	4.37, s	1c, 2c, 6c, 8c	74.9, CH_2	4.36, s	1c, 2c, 6c, 8c	6c
8c	58.8, CH_3	3.29, s	7c	58.6, CH_3	3.30, s	7c	6c
5c-OMe				61.1, CH_3	4.06, s	5c	6c

[a-f] Data are interchangeable within same character.

[g] Not detected because of a broad proton signal.

FDHRMS of odonthalol (**1)** suggests to be a tetrabrominated compound and its molecular formula of $C_{23}H_{20}Br_4O_8$. The ^{1}H NMR spectrum (Table 1) showed three aromatic, two methylenic, and two methoxy proton signals, along with a broad proton signal (δ_H 3.94). The ^{13}C NMR spectrum revealed eighteen aromatic, two methylenic and two methoxy carbon signals (Table 1). These signals were characterized to be an oligomer of BHB units. Unfortunately, HSQC and HMBC correlations of compound **1** were limited within individual two units of 1-methoxymethyl-2,3,4,5-tetrasubstituted benzene moieties. One remaining carbon signal was not determined because no correlation was observed with the broad proton signal of low signal height in HSQC and HMBC experiments. Thus little information of connection among different units was observed.

Compound **1** was converted with trimethylsilyldiazomethane in methanol to permethylated derivative **2**.[22] Molecular ion peak of derivative **2** increased 70 mass units compared to that of **1** in FDMS. New five methoxy signals were appeared in NMR spectra of **2** (Table 1). These data suggested presence of five free phenolic hydroxy groups in **1**. Therefore, one remaining oxygen atom would be used to form an ether linkage. The five methoxy groups were completely assigned to individual positions with consideration of HMBC and ROESY correlations (Figure 2). All other proton and carbon signals of derivative **2** except for carbon signals C-3a, C-3b and C-3c were also assigned from the 2D NMR data.

The three aromatic carbons signals at δ_C 122.7, 122.6, and 106.5, showing no correlation with the three aromatic proton signals in the HMBC experiment, are assigned to be the brominated aromatic carbons at *para*-positions (3a, 3b and 3c) of the three respective aromatic protons. Thus the remaining four aromatic carbons at the positions 2a, 2b, 7b, and 4c are suggested to connect among the three BHB units. Their carbon chemical shifts lead to determine combinations of connectivity, that is to say, alkylated aromatic C-2a (δ_C 129.1) is connected with alkylated aromatic C-1b (δ_C 130.5) via a methylene bridge at C-7b (δ_C 32.9). On the other hand, oxygenated aromatic C-2b (δ_C 142.6) is connected with oxygenated aromatic C-4c (δ_C 151.1) via an ether bridge. Thus structures of odonthalol (**1**) and its pentamethylated derivative (**2**) are determined as trimers of bromophenols. Partial structure of units A and B of odonthalol (**1)** had already isolated from the same alga.[16] Connectivity between units B and C of **1** may due to debrominated phenol coupling like the previously isolated bromophenols possessing a diaryl ether bridge.[10] To the best of our knowledge, odonthalol (**1**) is a novel algal bromophenol trimer whose BHB units are uniquely connected via ether and methylene bridges.

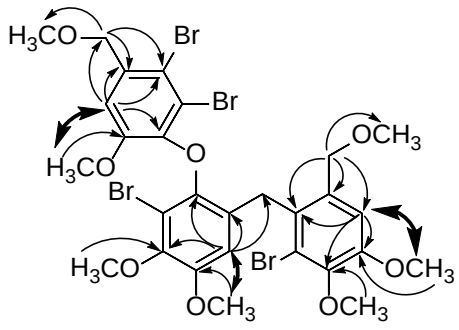


Figure 2. Key HMBC (single arrows) and ROESY (double arrows) correlations of compound **2**.

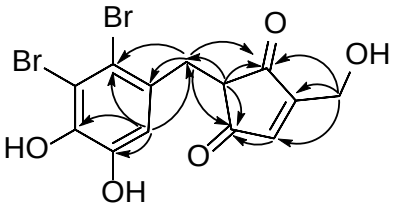


Figure 3. Key HMBC correlations of compound **3**.

Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) data of odonthadione (**3**) in acetone-d_6 and methanol-d_4

Position	δ_C in acetone-d_6	δ_H (J in Hz)	HMBC	δ_C in methanol-d_4	δ_H (J in Hz)
1a	131.1			131.0	
2a	116.4			116.6	
3a	113.6			114.2	
4a	144.1			144.7	
5a	145.2			146.0	
6a	118.0	6.89, s	2a, , 4a, 5a, 7a	117.9	6.76, s
7a	34.3	3.09, dd (14.6, 7.0) 3.05, dd (14.6, 7.0)	1a, 2a, 6a, 1b, 2b, 3b	34.9	3.07, dd (14.6, 7.0) 3.06, dd (14.6, 7.0)
1b	201.6			203.4[a]	
2b	51.3	3.22, t (7.0)	1a, 7a, 1b, 3b	51.8	NO[b]
3b	202.6			203.6[a]	
4b	166.2			166.7	
5b	143.0	7.10, s	1b	143.5	7.09, s
6b	57.3	4.51, s	3b, 4b, 5b	57.6	4.48, s

[a]Exchangeable.

[b]Not observed.

Odonthadione (**3)** was determined as a dibrominated compound and its molecular formula of $C_{13}H_{10}Br_2O_5$ based on FDHRMS data. The ^{1}H NMR spectrum in acetone-d_6 showed a conjugated olefinic proton at δ 7.10, an aromatic proton at δ 6.89, an equivalent methylene at δ 4.51, a non-equivalent methylene at δ 3.09/3.05, and a methine at δ 3.22 (Table 2). The last methine proton signal was disappeared in the ^{1}H NMR spectrum measured

in methanol-d_4. The ^{13}C NMR and HSQC spectrum revealed six aromatic, two ketonic, two olefinic, two methylenes, and one methine carbons. Among them, six aromatic and one methylene carbon are typical for the BHB unit. Both the methylene protons in BHB unit and methine proton were correlated not only one another but also with both ketonic carbons in HMBC experiment (Figure 3). However the olefinic and methylene protons were correlated with respective different ketonic carbons. Therefore, remaining structure other than the BHB unit was determined as 4-hydroxymethyl-4-cyclopentene-1,3-dion-2-yl moiety. Odonthadione (**3**) is not optically active under specific rotation measurement. This reason is seemed that compound **3** is racemic mixture because the proton at 2-position in 1,3-diketone moiety was easily exchangeable in protic solvent like the case of ^{1}H NMR spectrum in methanol-d_4. The compound **3** is a novel hybrid algal bromophenol, especially this is the first report on a naturally occurring cyclopentenedione moiety.

Table 3. Various activity[a] of odonthalol and odonthadione

Compound	DPPH EC_{50}[b] (μM)	ABTS EC_{50}[b] (μM)	CUPRAC $EC_{A0.50}$[c] (μM)	FRAP $EC_{A0.50}$[c] (μM)	Cu^{2+}-chelation EC_{50}[b] (μM)	Tyrosinase inhibition IC_{50}[d] (μM)
1	13.5±0.0	6.7±0.1	7.8±0.1	10.8±0.1	74.3±0.1	31.0±0.1
3	24.7±0.0	17.3±0.1	13.6±0.1	11.1±0.1	61.9±0.1	17.3±0.1
BHA	34.0±0.1	10.4±0.2	16.0±0.1	8.3±0.1		
Catechol	16.9±0.1	7.3±0.0	25.4±0.1	9.1±0.1		
EDTA					31.6±0.1	
Kojic acid						35.0±0.0

[a] All values are represented as mean ± standard error (n=3).

[b] The half maximal effect concentration.

[c] The effective concentration for absorbance of 0.50.

[d] The half maximal inhibitory concentration.

The isolated algal bromophenols **1** and **3** were investigated for antioxidant and tyrosinase inhibitory activities by employing free radical scavenging,[23,24] metal-reducing antioxidant,[25,26] copper chelation,[27] and tyrosinase inhibition[28] assays (Table 3). Bromophenols **1** and **3** exhibited almost same antioxidant activities compare with the positive controls examined. Compound **3** displayed two fold stronger tyrosinase inhibitory activities while compound **1** showed slightly higher activity with the positive control kojic acid. This study has disclosed new functionality of naturally occurring bromophenol as a tyrosinase inhibitor.

In conclusion, two new algal bromophenols showing tyrosinase inhibition and antioxidant were isolated from *O. corymbifera*. One is a BHB unit trimer which has an ether linkage. The other is a hybrid of a BHB unit and a unique cyclopentenedione moiety.

Acknowledgements

We express humble gratitude to Dr. E. Fukushi and Mr. Y. Takata, GC-MS and NMR Laboratory, Faculty of Agriculture, Hokkaido University for measuring mass and NMR spectra.

Supplementary Data

Supplementary data associated with this article can be found, in the online version, at XXX.

References and Notes

1. Kurihara H, Mitani T, Kawabata J, Takahashi K. *Fish Sci.* 1999; 65: 300-303.

2. Wang BG, Gloer JB, Ji NY, Zhao JC. *Chem Rev*. 2013; 113: 3632-3685.

3. Wiemer DF, Idler DD, Fenical W. *Experientia.* 1991; 47: 851-853.

4. Fan X, Xu NJ, Shi JG. *J Nat Prod*. 2003; 66: 455-458.

5. Combaut G, Chantraine JM, Teste J, Glombitza KW. *Phytochemistry.* 1978; 17: 1791-1792.

6. Li K, Li XM, Gloer JB, Wang BG. *Food Chem.* 2012; 135: 868-872.

7. Popplewell WL, Northcote PT. *Tetrahedron Lett.* 2009; 50: 6814-6817.

8. Xu XL, Piggott AM, Yin LY, Capon RJ, Song FH. *Tetrahedron Lett*. 2012; 53: 2103-2106.

9. Mikami D, Kurihara H, Ono M, Kim SM, Takahashi K. *Fitoterapia.* 2016; 108: 20-25.

10. Kurihara H, Mitani T, Kawabata J, Takahashi K. *J Nat Prod*. 1999; 62: 882-884.

11. Kim KY, Choi KS, Kurihara H, Kim SM. *Food Sci Biotechnol*. 2008; 17: 1110-1114.

12. Wang W, Okada Y, Shi H, Wang Y, Okuyana T. *J Nat Prod.* 2005; 68: 620-622.

13. Öztaskin N, Çetinkaya Y, Taslimi P, Göksu S, Gülcin I. *Bioorg Chem*. 2015; 60: 49-57.

14. Wang SY, Wang LJ, Jiang B , Wu N, Li XQ, Liu SF, Luo J, Shi DY. *Int J Mol Sci.* 2015; 16: 13548-13560.

15. Shi DY, Li XH, Li J, Guo SJ, Su H, Fan X. *Chin J Oceanol Limnol.* 2010; 28: 96-98.

16. Lee HS, Lee TH, Lee JH, Chae CS, Chung SC, Shin DS, Shin J, Oh KB. *J Agric Food Chem*. 2007; 55: 6923–6928.

17. Kurata K, Taniguchi K, Takashima K, Hayashi I, Suzuki M. *Phytochemistry*. 1997; 45: 485-487.

18. Oh KB, Lee JH, Chung SC, Shin J, Shin HJ, Kim HK, Lee HS. *Bioorg Med Chem Lett*. 2008; 18: 104-108.

19. Liu M, Wang GZ, Xiao L, Xu XL, Liu XH, Xu PX, Lin XK. *Mar Drugs*. 2014; 12: 3838-3851.

20. Odonthalol (**1**). Brown amorphous solid; UV (CH_3OH) λ_{max} (log ε) 210 (4.57), 232 sh (4.00), 290 (3.57) nm; IR ($CHCl_3$) ν_{max} 3528, 3022, 2928, 1718, 1602, 1281 cm^{-1}; ^1H NMR (500 MH_z, $(CD_3)_2CO$) and ^{13}C NMR (125 MHz, $(CD_3)_2CO$), see Table 1; FDMS *m/z* 740/742/744/746/748 (relative intensity 1:4:6:4:1); HRFDMS m/z 739.7888 (calcd for $C_{23}H_{20}{}^{79}Br_4O_8$, 739.7819).

21. Odonthadione (**3**). Light brown amorphous solid; $[\alpha]_D^{27}$ 0 (*c* 2.6, acetone); UV (CH_3OH) λ_{max} (log ε) 212 (4.28), 230 sh (3.94), 292 (3.32) nm; IR ($CHCl_3$) ν_{max} 3510, 3023, 2928, 2856, 1705, 1602, 1558, 1472 cm^{-1}; ^1H NMR (500 MH_z, $(CD_3)_2CO$) and ^{13}C NMR (125 MHz, $(CD_3)_2CO$), see Table 2; HRFDMS m/z 403.8889 (calcd for $C_{13}H_{10}{}^{79}Br_2O_5$, 403.8895).

22. Odonthalol (**1**) in MeOH was quantatively converted with trimethylsilyldiazomethane in hexane to pentamethyl ether (**2**) at room temperature. Odonthalol pentamethyl ether (**2**). Pale yellow amorphous solid; ^1H NMR (500 MH_z, $(CD_3)_2CO$) and ^{13}C NMR (125 MHz, $(CD_3)_2CO$), see Table 1; FDMS *m/z* 810/812/814/816/818 (relative intensity 1:4:6:4:1); HRFDMS m/z 809.8691 (calcd for $C_{28}H_{30}{}^{79}Br_4O_8$, 809.8675).

23. Guo Z, Li P, Hung W, Wang J, Liu Y, Liu B, Wang Y, Wu SB, Kennelly EJ, Long C. *Phytochemistry*. 2014; 106: 124-133.

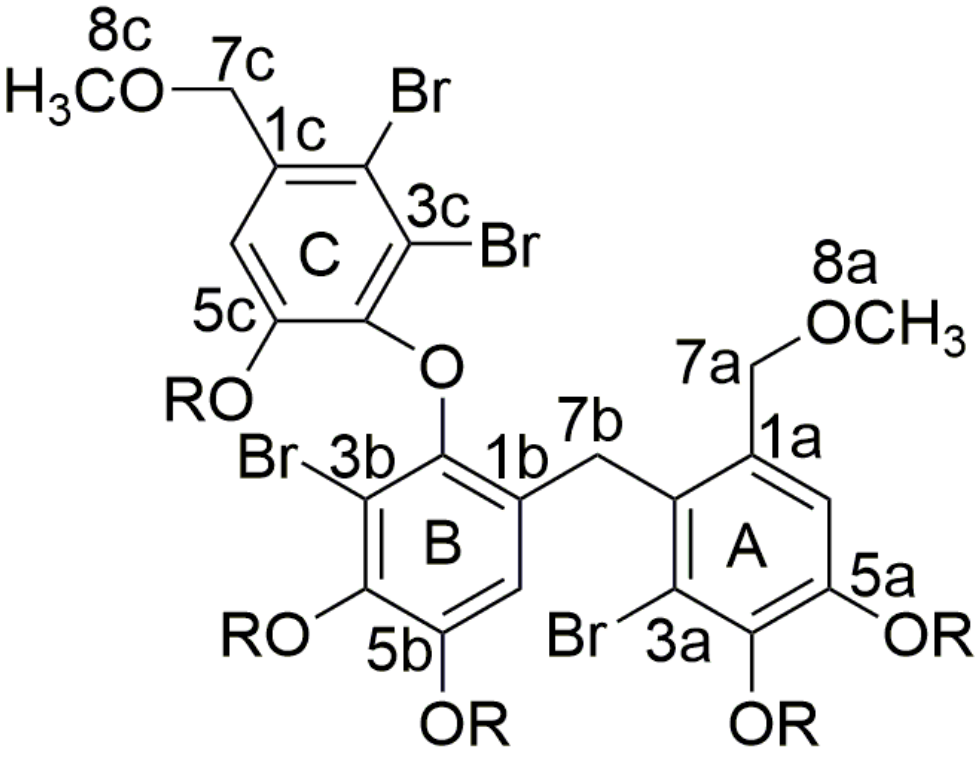
24. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Evans CR. *Free Radical Biol Med.* 1999; 26: 1231-1237.

25. Sabudak T, Demirkiran O, Ozturk M, Topcu G. *Phytochemistry.* 2013; 96: 305-311.

26. Benzie IFF, Strain JJ. *Anal Biochem.* 1996; 239: 70-76.

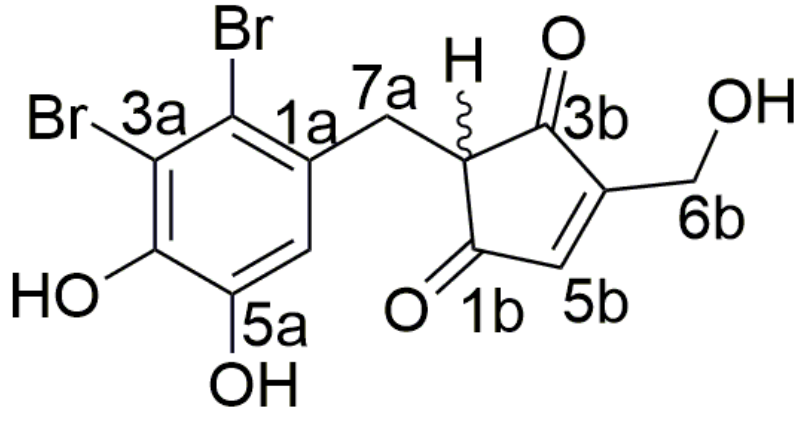
27. Santos JS, Brizola VRA, Granato D. *Food Chem*. 2017; 214: 515-522.

28. Zheng ZP, Cheng KW, Zhu Q, Wang XC, Lin ZX, Wang M. *J Agric Food Chem.* 2010; 58: 5368–73.

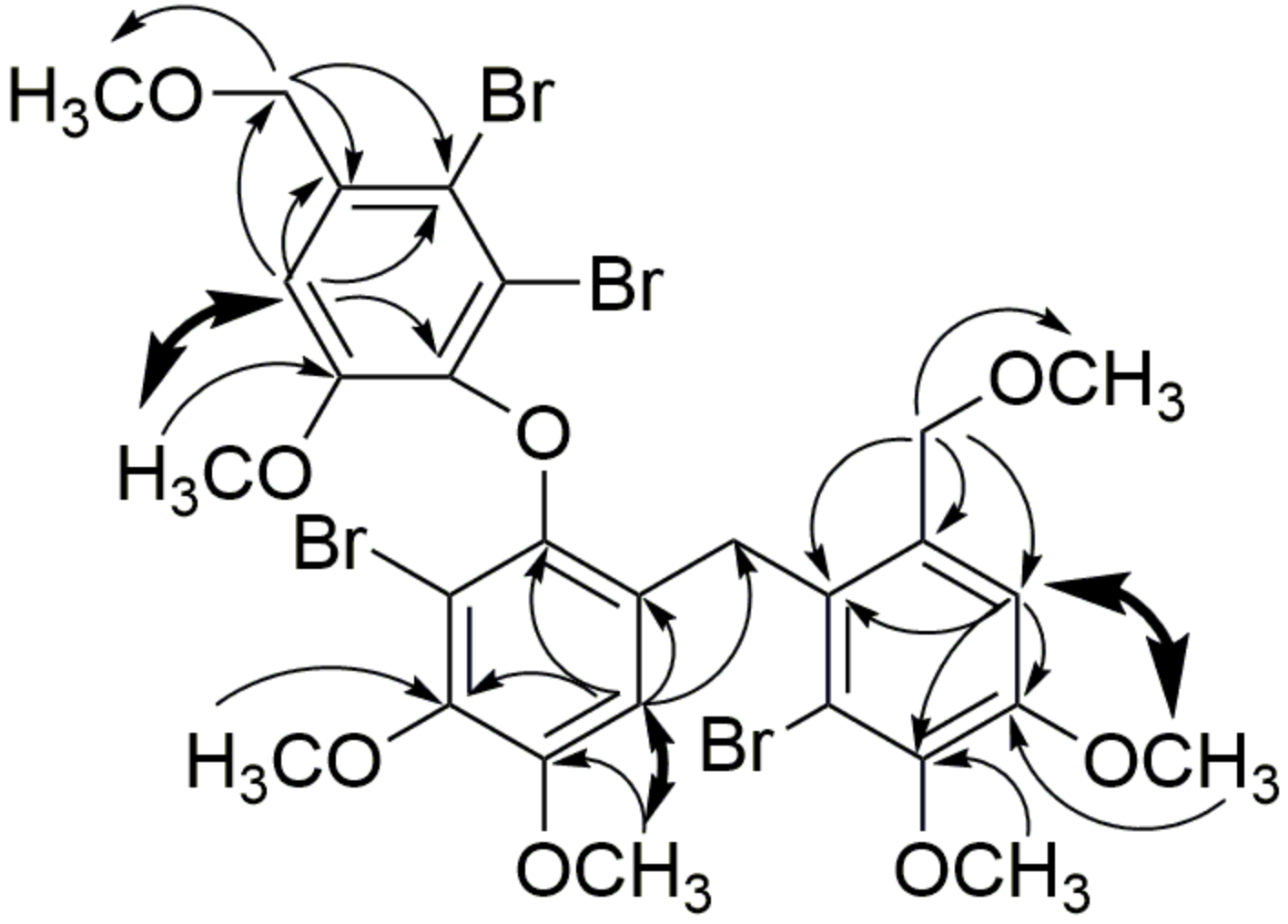


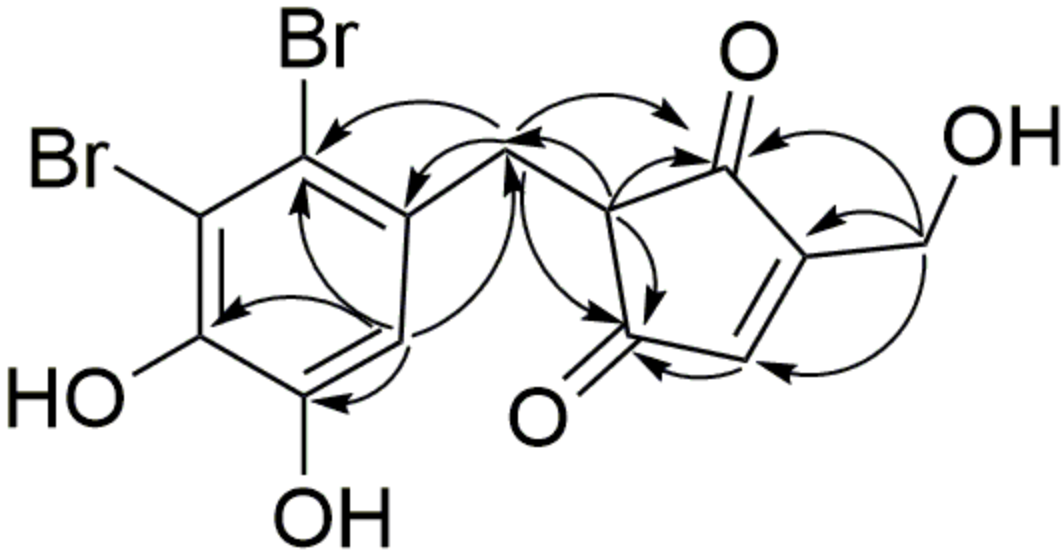
R=H Odonthalol (**1**)

R=Me **2**



Odonthadione (**3**)


H3CO
Br
Br
H3CO
O
Br
OCH3
H3CO
Br
OCH3
OCH3
OCH3


Br
Br
HO
OH
O
O
OH