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Urceolatin, a Structurally Unique Bromophenol from *Polysiphonia urceolata*

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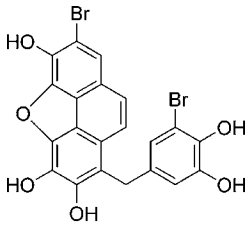
ABSTRACT



urceolatin (**1**)

6-Bromo-1-(3-bromo-4,5-dihydroxybenzyl)phenanthro[4,5-*bcd*]furan-2,3,5-triol (urceolatin, 1), a highly oxygenated bromophenol containing an unprecedented naturally occurring benzylphenanthro[4,5-*bcd*]furan unit, was isolated from the marine red alga *Polysiphonia urceolata*. Its structure was established on the basis of extensive spectroscopic analysis. Compound 1 displayed significant DPPH radical-scavenging activity with an IC_{50} value of 7.9 μM, which is 10-fold more potent than that of the positive control, butylated hydroxytoluene.

The marine red algae of the family Rhodomelaceae are a rich source of bromophenols. Previous phytochemical studies on species from this family have resulted in the characterization of a variety of bromophenols[1−5] with a range of biological activities, including feeding deterrent,[3] α-glucosidase inhibitory,[4] and growth stimulatory[5] effects. Recently, we initiated a program directed toward the discovery of structurally interesting and biologically active components from Chinese marine red algal species of the family Rhodomelaceae. Efforts to date have resulted in the isolation of a series of highly halogenated metabolites.[6−11] *Polysiphonia urceolata* is a member of the Rhodomelaceae family. We have recently reported that the crude extract, the EtOAc-soluble fraction, and the semipurified subfractions of *P. urceolata* possess potent DPPH radical-scavenging activity.[12] Bioassay-guided fractionation has resulted in the isolation

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and structure determination of a number of bromophenols from this species.[13,14]

In this paper, we report the isolation and structure elucidation of an unusual secondary metabolite from *P. urceolata* (urceolatin, **1**), which possesses a unique dibrominated benzylphenanthro[4,5-*bcd*]furan carbon skeleton.

A sample of the dried marine red alga *P. urceolata* (30.5 kg) was extracted with 95% EtOH at room temperature for 3 × 72 h. The crude extract was dissolved in H_2O and successively partitioned with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc-soluble extract (300 g) was subjected to column chromatography over Si gel (1200 g) eluting with petroleum ether−acetone (0−100%) and $CHCl_3$−MeOH (10−100%) to afford 36 fractions on the basis of TLC analysis. The fraction eluted with $CHCl_3$−MeOH (5:1) was further chromatographed over Si gel eluting with a gradient of increasing acetone (50−100%) in petroleum ether to yield three subfractions. The third subfraction (petroleum ether/acetone 7:3, v/v) was further purified by reversed-phase semipreparative HPLC using MeOH−H_2O (30:70) as the mobile phase to yield urceolatin **1** (7.9 mg).

Urceolatin (**1**)[15] was obtained as a red amorphous powder. The IR spectrum displayed hydroxy (3400 cm^{-1}) and aromatic ring (1642 and 1584 cm^{-1}) absorptions. The UV spectrum showed intense absorptions consistent with an extended aromatic system. The EIMS gave a characteristic dibrominated molecular ion peak cluster at *m*/*z* 522/520/518 (1:2:1) $[M]^+$, and the molecular formula was determined by HRESIMS[15] to be $C_{21}H_{12}Br_2O_6$, implying 15 degrees of unsaturation. The ^{1}H NMR spectrum (acetone-d_6, Table 1) contained one aromatic singlet at δ 7.98, two *ortho*-coupled aromatic doublets at δ 7.82 and 7.69, two *meta*-coupled aromatic doublets at δ 6.99 and 6.76, and a two-proton singlet at δ 4.38. In accordance with the molecular formula, 21 carbon signals were observed in the ^{13}C NMR spectrum (Table 1) and were classified by DEPT experiments as 1 upfield-shifted sp^3 methylene (δ 31.1, C-7′), 5 sp^2 methines, and 15 nonprotonated sp^2 carbons (Table 1). The oxygenated (C-2, C-3, C-4, C-5, C-6, C-4′, and C-5′) and brominated (C-7 and C-3′) aromatic carbons were recognized by their chemical shifts at lower (δ > 135) and higher (δ < 120) fields, respectively.[8] Comparison of the DEPT data with the molecular formula required **1** to contain five exchangeable hydrogens, all of which must be present as phenolic OH groups. One ether linkage should also be present to account for the remaining two oxygenated carbons and the sixth oxygen atom present in the formula. Unfortunately, well-defined OH signals were not observed in any of the ^{1}H NMR spectra recorded using various solvents.

Table 1. ^{1}H and ^{13}C NMR Data for Compound **1**[b]

no.	δ_C	no.	δ_H (*J* in Hz)	δ_C
1	120.5	8	7.98 (s)	124.2
1a	119.2	8a		118.9
2	147.9	9	7.82 (d, 9.0)	123.1
3	141.2	10	7.69 (d, 9.0)	123.0
4	135.2[a]	1′		135.2
4a	121.6	2′	6.99 (d, 1.5)	124.1
5	136.2[a]	3′		109.9
5a	126.1	4′		141.7
6	142.2	5′		146.5
7	113.0	6′	6.76 (d, 1.5)	115.7
		7′	4.38 (s)	31.1

[a] These assignments are interchangeable. [b] Acetone-d_6, 500 MHz for ^{1}H, 125 MHz for ^{13}C, δ in ppm.

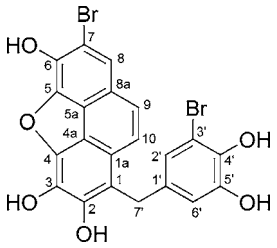

Figure 1. The structure of urceolatin (**1**).

In the HMBC spectrum, one of the two *meta*-coupled aromatic protons (H-2′) displayed long-range correlations to C-4′, C-6′, and C-7′, while the other (H-6′) exhibited correlations to C-2′, C-4′, and C-7′. In addition, the two-proton singlet for H_2-7′ (δ 4.38) displayed HMBC correlations to C-2′ and C-6′ (Figure 2). These data as well as the corresponding chemical shift values indicated the presence of a 3-bromo-4,5-dihydroxybenzyl moiety, which was supported by the similarity of the ^{1}H and ^{13}C NMR data to those reported in the literature for such a unit.[2,16] An observed EIMS fragment ion peak cluster at *m*/*z* 201 and 203 (in a 1:1 ratio) reinforced this deduction.

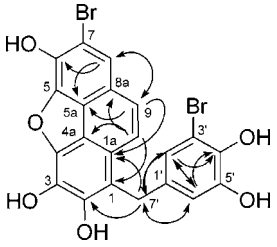

Figure 2. Key HMBC correlations for urceolatin (**1**).

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(15) Urceolatin (**1**): red amorphous powder; UV λ_{max} (MeOH) (log ϵ) 205 (4.47), 234 (4.23), 267 (4.48), 335 (3.86) nm; IR (KBr) ν_{max} 3400, 2925, 2852, 1642, 1584, 1496, 1433, 1383, 1297, 1269, 1220, 1165, 1111, 1061, 1084, 994, 958, 922, 849, 806, 782 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; EIMS *m*/*z* 522 (2), 520 (4), 518 (2) $[M]^+$, 442 (4), 440 (4) $[M - Br]^+$, 361 (10) $[M - 2Br]^+$, 343 (12), 208 (58), 203 (8), 201 (9), 82 (100); HRESIMS *m*/*z* 542.8890 ($[M + Na]^+$, calcd for $C_{21}H_{12}{}^{79}Br^{81}BrO_6Na^+$, 542.8878).

The remaining portion of **1** should possess the elemental composition $C_{14}H_6BrO_4$ and has to account for the remaining 11 degrees of unsaturation. HMBC correlations from H-9 to C-1a, C-5a, and C-8, from H-10 to C-1, C-4a, and C-8a, and from H-8 to C-5a, C-6, and C-9, in combination with the chemical shift values of the corresponding carbons and the elemental composition, resulted in the assignment of a phenanthrene structural unit containing one brominated and five oxygenated carbons placed as shown in Figure 2. Comparison of the remaining elemental composition ($C_{14}H_6BrO_4$, $\Omega = 11$) with the proposed substituted phenanthrene unit ($C_{14}H_8BrO_5$, $\Omega = 10$) resulted in the conclusion that C-4 and C-5 of the phenanthrene moiety are linked via an oxygen atom to account for the required ether linkage and the remaining degree of unsaturation, thereby forming a phenanthro[4,5-*bcd*]furan moiety.[17]

Further HMBC correlations from H_2-7′ to C-1a and C-2 (δ 147.9, s) allowed attachment of the 3-bromo-4,5-dihydroxybenzyl unit to C-1 of the phenanthro[4, 5-*bcd*]furan moiety to complete the structure of **1** as shown. Thus, the structure of **1** was assigned as 6-bromo-1-(3-bromo-4,5-dihydroxybenzyl)phenanthro[4,5-*bcd*]furan-2,3,5-triol, for which the name urceolatin is proposed.

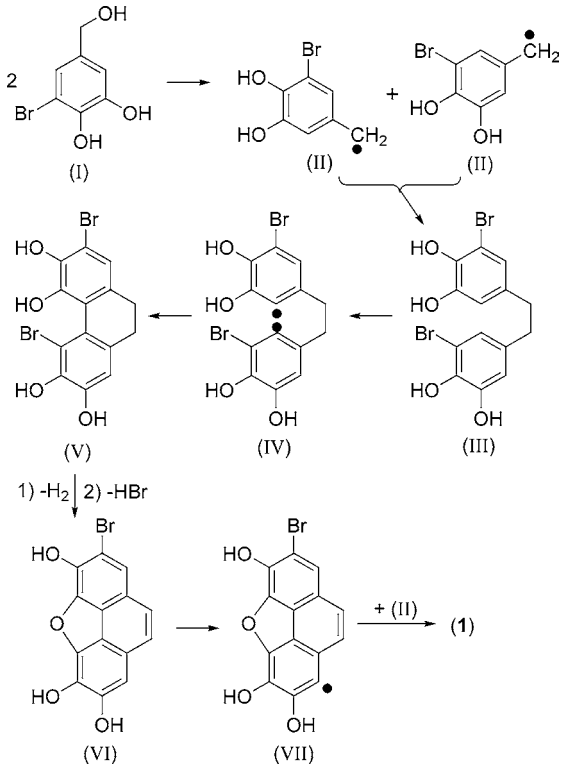
The three-dimensional (3D) structure of **1**, as simulated using Cambridgesoft Chem3D Ultra (version 8.0) with an MM2 force field calculation for energy minimization, suggests that the molecule prefers to adopt a conformation in which the plane containing the phenanthrene and furan rings is nearly perpendicular to that of the substituted benzyl group ring.

Compound **1** was evaluated for the ability to scavenge free radicals of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) by using a previously reported procedure.[12] It displayed significant activity, with an IC_{50} value of 7.9 μM, which is 10.6-fold more potent than that of butylated hydroxytoluene (BHT), a well-known synthetic positive control (which has an IC_{50} value of 83.8 μM).

A synthetic method for the preparation of phenanthro[4,5-*bcd*]furan has been previously reported,[17] and a compound containing such a unit (morphenol) is known as a degradation product of morphine. Phenanthrene and dihydrophenanthrene derivatives have been isolated from the terrestrial plants *Bletilla striata*,[18−20] *Pleione bulbocodioides*,[21] and *Spiranthes sinensis*,[22] all belonging to the plant family Orchidaceae. However, to the best of our knowledge, there are no prior reports of natural products containing a phenanthro[4,5-*bcd*]-furan moiety. This may be due in part to the presence of ring strain in the phenanthro[4,5-*bcd*]furan unit.[17]

From a biogenetic point of view, compound **1** is likely generated by intermolecular free radical reactions[23] involving three molecules of 3-bromo-5-(hydroxymethyl)-benzene-1,2-diol (I, Scheme 1) or involving one molecule

Scheme 1. Possible Biogenesis of Compound **1**



of I and one molecule of 1,2-bis(3-bromo-4,5-dihydroxyphenyl)ethane (III, Scheme 1**)**, followed by additional intramolecular coupling through a biradical (IV) to yield the intermediate dihydrophenanthrene (V), which, by a series of reactions such as dehydrogenation and loss of HBr, forms another intermediate containing a phenanthro[4,5-*bcd*]-furan ring system (VI). A radical (VII) generated from VI could then further couple with radical II to give compound **1**. Both of the putative precursors 3-bromo-5-(hydroxymethyl)benzene-1,2-diol (I) and 1,2-bis(3-bromo-4,5-dihydroxyphenyl)ethane (III) have been isolated from *P. urceolata* in our laboratory as well as from some other marine red algae.[13,14]

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Supporting Information Available: Experimental procedures, details regarding the algal sample, 1D (^{1}H, ^{13}C, and DEPT) and 2D (^{1}H−^{1}H COSY, HSQC, and HMBC) NMR spectra, MS data, and the HPLC-UV profile for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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