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ARTICLE

Antimicrobial agent isolated from *Coptidis rhizome* extract incubated with *Rhodococcus* sp. strain BD7100

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Abstract

Coptidis rhizome (CR) is a widely used herbal medicine that contains protoberberine-type alkaloids. CR extract exhibits various pharmacologic activities. A previous study reported the isolation of *Rhodococcus* sp. strain BD7100 as a berberine (BBR)-utilizing bacterium, and the BBR-degradation pathway has been investigated. When we incubated strain BD7100 cells with CR extract, the number of viable cells declined with the degradation of components in the CR extract, and the culture broth exhibited antibacterial activity against strain BD7100. These results suggest that CR extract cultured in the presence of strain BD7100 contains one or more antibacterial agents. In this study, we isolated coptirhoquinone A (**1**) from CR extract incubated with strain BD7100 in Luria–Bertani (LB) medium, and the structure was elucidated using NMR and MS analysis. We also report the total synthesis and antimicrobial activities of **1** against bacteria, fungi, and *Pythium* sp.

Introduction

Coptidis rhizome (CR) is the dried rhizome of plants of the genus *Coptis*, such as *C. japonica* Makino and *C. chinensis* Franch. CR is widely used as a herbal medicine to relieve abdominal pain and diarrhea in traditional Chinese medicine and Japanese Kampo [1, 2]. Previous studies reported that CR extract and traditional medicines containing CR exhibit antimicrobial, antimalarial, anti-inflammatory, and analgesic activities [3–6]. CR contains protoberberine-type alkaloids such as berberine (BBR), palmatine (PAL), coptisine (COP), jatrorrhizine (JAT), *epi*-berberine (E-BBR), and columbamine (COL) [7].

We have been investigating bacterial degradation of alkaloids. *Rhodococcus* sp. strain BD7100 is a bacterium isolated from soil that can utilize BBR as a sole carbon source [8]. A recent report indicated that strain BD7100 degrades BBR into 2-hydroxy-3,4-dimethoxybenzeneacetic acid (HDBA) via demethylenation to D-BBR [9]. In this work, we investigated the degradation of protoberberine-type alkaloids by strain BD7100 by incubating the bacterium with CR extract, which contains numerous protoberberine-type alkaloids. Unexpectedly, we found that the number of viable cells of strain BD7100 declined, and the culture medium exhibited antimicrobial activity against strain BD7100 that increased in potency as the level of protoberberine-type alkaloids decreased. These results suggested that an antibacterial agent is produced in CR extract incubated with strain BD7100. In this study, we report a novel antimicrobial agent was being produced from the CR extract when incubated strain BD7100 in Luria–Bertani (LB) medium. We also elucidated the structure of the antimicrobial agent using NMR and MS analysis and report its total synthesis and spectrum of activity.

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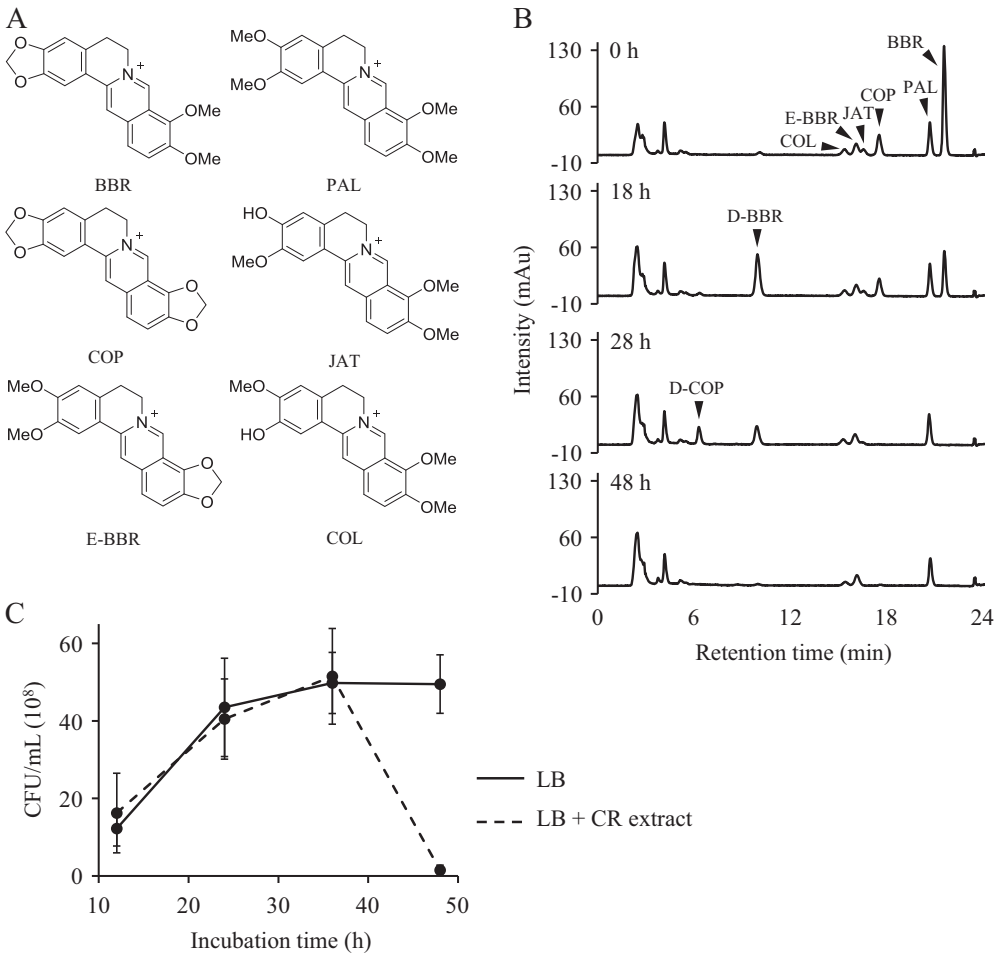


Fig. 1 Incubation of CR extract with *Rhodococcus* sp. strain BD7100 in LB medium. **a** Chemical structures of protoberberine-type alkaloids identified in CR extract. **b** HPLC chromatograms ($\lambda = 280$ nm) of CR extract incubated with cells of strain BD7100. **c** Change in the number of viable bacteria. Solid line indicates the number of viable bacteria in LB medium, and dotted line indicates the number of viable bacteria in LB medium containing CR extract. Each value represents the mean ± SD of four independent experiments

Table 1 Time course of antibacterial effect of culture medium against strain BD7100, as determined using the paper-disk method

	Incubation time (h)					
Bacterium	0	12	24	32	36	48
BD7100	n.d.	n.d.	n.d.	n.d.	15	12.5

Amount of sample: 50 µL/disc (8 mm), unit: mm

Results and Discussion

Production of antimicrobial agent from CR extract by *Rhodococcus* sp. strain BD7100 in LB medium

CR extract was added to LB medium and incubated with cells of strain BD7100. After 48 h, the culture broth was analyzed using HPLC. BBR, PAL, COP, JAT, E-BBR, and COL were detected in samples analyzed by HPLC at the beginning of the incubation period (Fig. 1a, b). HPLC chromatograms indicated that BBR, COP, and JAT were degraded during incubation, and demethyleneberberine and demethylenecoptisine were produced. BBR, COP, and JAT were completely degraded by 48 h, whereas PAL, E-BBR, and COL were still detected after 48 h (Figure S1).

The number of viable BD7100 cells in culture broth with and without CR extract was determined using the dilution-plate method (Fig. 1c). The viable cell counts exhibited similar trends until 36 h. In culture broth containing CR extract, the number of viable cells decreased markedly by 48 h. Antibacterial activity in culture broth containing CR extract was tested against strain BD7100 using the paper-disk method. At 36 and 48 h, culture broth exhibited antibacterial activity against BD7100 cells; however, there was no activity observed at other time points (Table 1). These results suggested that one or more antimicrobial compounds active against strain BD7100 is produced in CR extract incubated with BD7100 cells. Then we tried to bioassay-guided isolation to identify bioactive compounds.

Elucidation of the structure of coptirhoquinone A (1)

Compound **1** was isolated as a red, amorphous solid, and the molecular formula was determined as $C_{12}H_{14}O_6S$ (six degrees of unsaturation) by high-resolution electrospray ionization mass spectrometry (HRESIMS). IR spectra (1740, 1716, and 1653 cm^{-1}) and ^{13}C NMR signals indicated the presence of two α, β-unsaturated ketones (δ_C 181.1 and 180.2) and an ester bond (δ_C 169.7) in the structure of **1**. ^1H NMR spectra of **1** exhibited signals for three methyl groups with an oxygen atom (δ_H 4.01, 4.00, and 3.70), a methyl group with a sulfur atom (δ_H 2.56), and a methylene group (δ_H 3.77). A heteronuclear multiple bond coherence (HMBC) correlation of 8-OCH_3 [δ_H 3.70 (s)] with C-8 (δ_C 169.7) indicated the presence of a methyl ester residue. HMBC correlations of 3-OCH_3 (δ_H 4.01 [s]) with C-3 (δ_C 145.4) and 4-OCH_3 (δ_H 4.00 [s]) with C-4 (δ_C 144.9) indicated linkages between two methoxy groups at the C-3 and C-4 positions. An HMBC correlation of 6-SCH_3 (δ_H 2.56 [s]) with C-6 (δ_C 145.6) indicated a linkage between a sulfurated methyl group and the C-6 position. From these results, the structure of **1** was determined as shown in Fig. 2 and Table 2.

Total chemical synthesis of coptirhoquinone A (1)

Total synthesis of compound **1** was carried out to confirm its structure. Benzylation of known phenol **4** [10, 11] (readily derived from 2,3,4-trimethoxylbenzaldehyde in three steps) with benzyl bromide in the presence of K_2CO_3 afforded benzyl ether **5** at 94% yield (Scheme 1). Rearrangement of **5** with HMBI (**7**) (1*H*-1-hydroxy-5-methyl-1,2,3-benziodoxanthiole 3,3-dioxide) [12] and trimethyl orthoformate was carried out at room temperature for 2 h to furnish the methyl acetate **6** at 66% yield [13]. Bromination of **6** with NH_4Br-oxone [14] brought about the expected bromination to give the desired product **8** at 67% yield. Hydrogenation of **8** with Pd/C, followed by oxidation of the resulting phenol **9** with nBu_4NBr-oxone [15], gave quinone **10** at 76% yield over two steps. Finally, substitution of **10** with sodium thiomethoxide [16] gave the desired product **1** at 76% yield. The spectroscopic data of synthesized **1** were superimposable with those of the natural product. Thus, we accomplished the total synthesis of **1** in six steps from **4** at 16.3% overall yield (Scheme 1).

Antimicrobial activity of coptirhoquinone A (1)

The antibacterial activity of **1** against strain BD7100 and various intestinal bacteria was tested using the paper-disk method (Table 3). In the presence of disks permeated with **1**, growth of strain BD7100 was inhibited at 0.25 mg per disk (25.7 mm), similar to the same concentrations of ampicillin and kanamycin (25 and 24.7 mm, respectively). Compound **1** also inhibited the growth of *Staphylococcus aureus* (32.7 mm) and *Proteus mirabillis* (16.3 mm) at 0.25 mg per disk. No inhibition of the growth of *Escherichia coli*, *Klebsiella neumoniae*, or *Pseudomonus aerginosa* was observed.

Compound **1** was also tested for activity against pathogens associated with deep mycosis, such as *Aspergillus fumigatus*, *A. niger*, *Candida albicans*, and *Cryptococcus neoformans*. Compound **1** inhibited the growth of *A. fumigatus* (12.3 mm), *C. albicans* (10.3 mm), and *C. neoformans* (12.7 mm).

Compounds consisting of a benzoquinone skeleton have been reported to exhibit antibacterial activity against a variety of microorganisms [17, 18], and synthesis of various derivatives has been reported [19]. In particular, benzoquinone derivatives containing a methylthio group

Table 2 NMR chemical shifts of coptirhoquinone A (**1**) and synthetic compound in $CDCl_3$

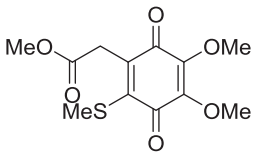
	1			Synthetic compound	
Position	δ_C[a]	δ_H[b]	HMBC[c]	δ_C[a]	δ_H[b]
1	137.9	–		138.0	–
2	181.1	–		181.0	–
3	145.4	–		145.4	–
4	144.9	–		144.9	–
5	180.2	–		180.1	–
6	–		145.6	–	
7	33.7	3.77 s	1, 2, 6, 8	33.7	3.76 s
8	169.7	–		169.7	–
3-OCH_3	61.5 *	4.01 s	3	61.5*	4.01 s
4-OCH_3	61.4 *	4.00 s	4	61.4*	4.00 s
6-SCH_3	17.9	2.56 s	6	17.9	2.56 s
7-OCH_3					
8-$COOCH_3$	52.5	3.70 s	8	52.5	3.70 s

[a]100 MHz for ^{13}C-NMR

[b]400 MHz for ^1H-NMR

[c]HMBC correlations are from the proton(s) stated to the indicated carbon

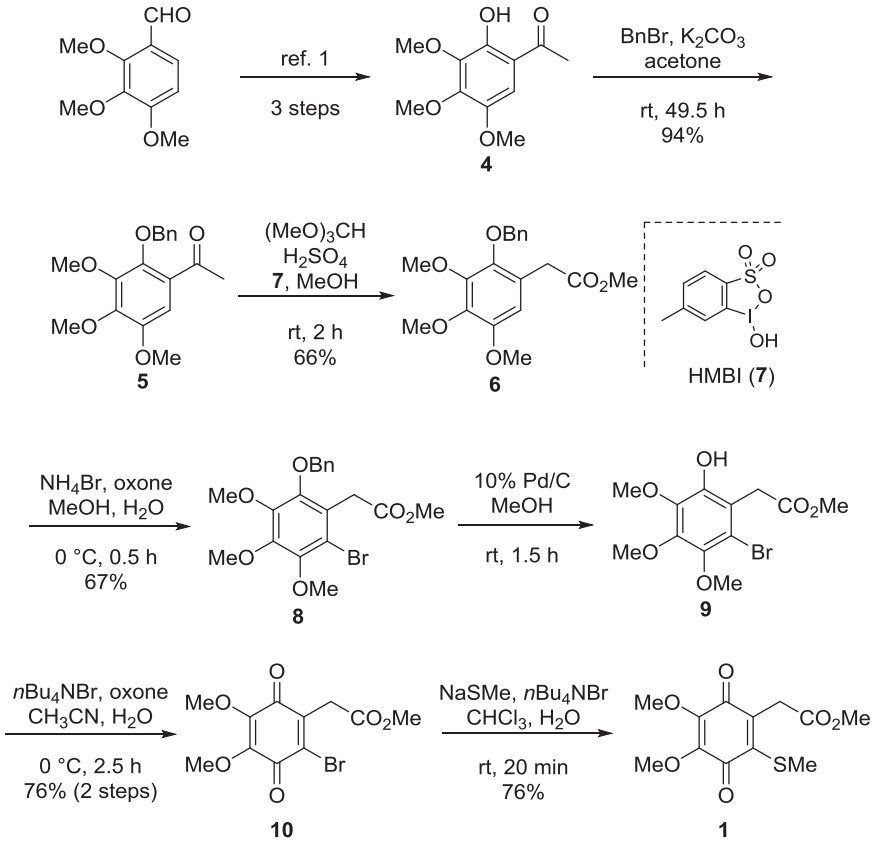
*Exchangeable



1

Fig. 2 Structure of **1**

Scheme 1 Total synthesis of **1** via rearrangement reaction



reportedly exhibit enhanced antibacterial activity. However, the isolation of natural benzoquinone derivatives containing methylthio groups is rare; examples include T1801B, isolated from the bacterium *Pseudomonas* [20], and thiographisquinone, isolated from Graphis [21], both of which consist of a 2-methylthio-3,4-benzoquinone skeleton. T1801B reportedly exhibits antibacterial activity against *S. aureus* and *C. neoformans*.

In this study, a novel antimicrobial substance was produced by incubating CR extract with a BBR-utilizing bacterium. These results suggest that it would be possible to more effectively utilize resources by preparing CR extract from waste generated during the manufacture of herbal medicine preparations and using it as a raw material. In addition, this study serves as a model for the preparation of novel compounds by the biotransformation of herbal extracts, and we hypothesize that the results could be applied to other herbal extracts and bacteria.

Materials and methods

Experimental instruments

UV and IR spectra were recorded on an Ultrospec 2100 pro UV-visible spectrophotometer (Amersham Biosciences, Ltd., Tokyo, Japan) and a FT/IR-4100 instrument (JASCO Co., Ltd., Tokyo, Japan), respectively. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker AVANCE-400 spectrometer (400.13 MHz for ^{1}H, 100.61 MHz for ^{13}C; Bruker Biospin K.K., Kanagawa, Japan). Chemical shifts (δ) were measured in ppm using tetramethylsilane as an internal standard. BioShakers BR43-FH (TAITEC Co., Ltd., Saitama, Japan) and FTW-502M (Hirayama Manufacturing Corp., Saitama, Japan) were used for incubation of bacteria. The turbidity of cultures was monitored using an OD-MonitorA&S (TAITEC Co.). Samples were analyzed and quantified by PDA-HPLC using PU-980 and PU-1580 pumps (JASCO Co.) equipped with an Inertsil ODS-3 column (5 µm, 4.6 mm × 250 mm; GL Science Inc., Tokyo, Japan; maintained at a temperature of 30 °C using a CO-2065 Plus column oven [JASCO Co.]) and an MD-2010 Plus photodiode array detector (JASCO Co.). Compounds were isolated using a reverse-phase MPLC system equipped with an SSC-3160 pump (Senshu Scientific Co., Ltd., Tokyo, Japan), ULTRA PACK ODS-SM-50A column (300 mm × 11 mm [50 µm], Yamazen Corp., Osaka, Japan), and YRU-8883 RI-UV detector (Shimamuratech, Tokyo, Japan). Reverse-phase HPLC was also carried out using an LC-20AT prominence pump (Shimadzu Corp., Kyoto, Japan) equipped with an Inertsil ODS-3 column (250 mm × 4.6 mm [5 µm], GL Science Inc.), CO-965 column oven

Table 3 Antimicrobial activity of **1** and various antibiotics, as determined using the paper-disk method

Microorganisms	1	Amp	Kan	AmB
Bacterial sp.				
Rhodococcus sp. BD7100	25.7 ± 0.6	25.0 ± 1.0	24.7 ± 2.5	–
Staphylococcus aureus	32.7 ± 3.2	47.7 ± 11.0	40.7 ± 2.9	–
Escherichia coli	0.0 ± 0.0	29.0 ± 1.0	27.3 ± 0.6	–
Klebsiella neumoniae	8.0 ± 7.0	14.7 ± 0.6	18.3 ± 9.0	–
Proteum mirabillis	16.3 ± 0.6	34.3 ± 2.1	26.7 ± 1.5	–
Pseudomonas aeruginosa	0.0 ± 0.0	0.0 ± 0.0	14.7 ± 0.6	–
Fungal sp.				
Aspegillus fumigatus	12.3 ± 0.6	–	–	13.3 ± 0.6
Aspegillus niger	0.0 ± 0.0	–	–	14.7 ± 1.5
Candida albicans	10.3 ± 0.6	–	–	17.3 ± 6.4
Cryptococcus neoformans	12.7 ± 1.2	–	–	19.3 ± 0.6

Amp ampcillin, *Kan* kanamycin, *AmB* amphotericin B, *n.d.* not detected, – not tested

Each value represents the mean ± SD of three independent experiments ($n = 3$)

(JASCO Co.), and SPD-20A UV detector (Shimadzu Corp.). Molecular weight and molecular formula were determined by LC-ESI-TOF-MS using a JMS-T100LP spectrometer (JEOL, Ltd., Tokyo, Japan) and 1200 series system (Agilent Technologies, Inc., Tokyo, Japan).

Strains and culture conditions

The isolation and identification of *Rhodococcus* sp. strain BD7100 has been described previously [9]. For the present work, strain BD7100 was incubated in LB medium (0.5 g Bacto yeast extract, 1.0 g Bacto tryptone, and 0.5 g NaCl in 100 mL of water) or on 0.1 × LB agar medium (containing Bacto yeast extract and Bacto tryptone at 10-fold lower levels and 1.5% [w/v] agar). CR extract was added to these media as indicated.

Preparation of CR extract

Dried *Coptidis rhizome* (100 g, Uchida Wakan-yaku, Tokyo, Japan) was boiled in water (590 mL) at 100 °C for 30 min, and the extract was then filtered directly. The filtrate was centrifuged (2610 × *g*, r.t., 15 min), and the supernatant was filtered using a sterilized 0.2-μm filter. The resulting filtrate was used as the CR extract.

Incubation of CR extract with strain BD7100 in LB medium

Rhodococcus sp. strain BD7100 was grown at 30 °C on 0.1 × LB agar plates containing 1.0 mM BBR. Individual colonies of strain BD7100 were used to inoculate 10 mL of LB medium in a test tube, which was incubated for 24 h at 30 °C on a rotary shaker (180 rpm). After incubation, the culture was inoculated into LB medium (100 mL) containing CR extract (5 mL) in a 500-mL Erlenmeyer flask rotated at 180 rpm. Next, 0.5-mL samples of the culture were collected at various time points and mixed with an equal amount of methanol (MeOH) containing 0.4% (v/v) hydrochloric acid. This mixture was centrifuged (18,800 × *g*, 4 °C, 5 min) to remove cells and debris.

PDA-HPLC analysis

The flow rate was 1 mL/min, and the column oven temperature was 30 °C. Analysis was performed using a mobile phase gradient as follows. Initial conditions were 15% for 5 min, followed by a linear gradient to 50% B from 5 to 15 min and 100% B at 15.1 min, which was maintained until 18 min. The mobile phase was returned to initial conditions at 18.1 min and maintained until the end of the run at 25 min. Mobile phase solvents A and B were H_2O containing 0.1% trifluoroacetic acid and acetonitrile, respectively.

Determination of the number of viable bacteria

Rhodococcus sp. strain BD7100 was incubated in flasks (500 mL) containing 100 mL of LB medium with or without 5 mL of CR extract at 30 °C with rotation (180 rpm). Samples (100 μL) of the cultures were collected at 12, 24, 36, and 48 h of incubation and diluted 10^7 fold using sterilized water. Aliquots (100 μL) of the diluted cultures were inoculated onto LB agar plates, which were incubated at 30 °C for 48 h. After incubation, colonies on the plates were counted.

Investigation of antibacterial activity in cultures containing CR extract

Strain BD7100 was incubated in LB medium containing CR extract. The incubation conditions were the same as those described above. After incubation, 20 mL of each culture

was removed, frozen, and lyophilized. Next, 1 mL of MeOH containing 0.4% (v/v) hydrochloric acid was added to each sample and centrifuged (2610 × *g*, r.t., 10 min). The resulting supernatant was used to test antibacterial activity using the paper-disk method.

Testing antibacterial activity using the paper-disk method

A 50-μL aliquot of sample was applied to an 8-mm disk. The test samples were dissolved in MeOH containing 0.4% (v/v) hydrochloric acid. The disks containing sample were placed on an LB agar plate inoculated with bacteria, and the plate was incubated at 30 °C for 12 or 24 h. After incubation, the diameter of inhibitory zone around each disk was measured.

Isolation of compound coptirhoquinone A (1)

Rhodococcus sp. strain BD7100 was incubated in 36 flasks containing LB medium (100 mL) with CR extract (5 mL) at 30 °C for 48 h. The culture was then centrifuged (2610 × *g*, r.t., 15 min) to remove cells, and the resulting supernatant was extracted using two volumes of ethyl acetate, two volumes of ethyl acetate containing 0.08% (v/v) hydrochloric acid, and an equal volume of ethyl acetate. The ethyl acetate was evaporated, and the residue (2.08 g) was suspended in water. The suspension was extracted twice with chloroform, and then the chloroform was evaporated. The chloroform extract (571.8 mg) was dissolved in MeOH, and the solution was fractionated using a reverse-phase MPLC system at a flow rate of 5 mL/min and the following gradient: initial conditions, 0% B (0–30 min); 15% (30–75 min), 30% B (75–105 min), 50% (105–125 min), 70% B (125–160 min), 100% (160–180 min) (A: H_2O, B: acetonitrile). The sixth fraction was chromatographed to isolate and purify compound **1** (3.0 mg) using reverse-phase HPLC under the following conditions: flow rate, 1.0 mL/min; column oven temperature, 30 °C; detector, UV at 210 nm; mobile phase, 65% H_2O containing 0.1% (v/v) trifluoroacetic acid and 35% acetonitrile.

Compound **1**: Red, amorphous solid; HRESIMS obsd. 287.0601 calcd. for $C_{12}H_{15}O_6S_1$ $[M+H]^+$ 287.0589; UV λ_{max} (MeOH) nm (log ε): 206 (4.0), 270 (3.5), 342 (3.4); IR (KBr) cm^{-1}: 1740, 1716, 1653. The NMR data see Table 2.

General methods

Melting points were determined using a Yanagimoto MP apparatus, and the values are uncorrected. Optical rotations were measured using a JASCO DIP-360. IR spectra were obtained using a JASCO FT/IR-4100 spectrophotometer. ^1H and ^{13}C NMR spectra were obtained on a Bruker AV III 400 (^1H-NMR: 400 MHz, ^{13}C NMR: 100 MHz) instrument for solutions in $CDCl_3$, and chemical shifts are reported on the δ scale using TMS as an internal standard of 0.00 for ^1H NMR spectra and $CDCl_3$ as an internal standard of δ 77.00 for ^{13}C NMR spectra. MS spectra were obtained on a JEOL-600 spectrometer. Elemental analyses were performed on a Yanaco-MT5.

2,3,4-Trimethoxyphenol

To a solution of 2,3,4-trimethoxybenzaldehyde (1.0 g, 5.1 mmol) in MeOH (7.3 mL), 31% H_2O_2 (0.75 mg, 6.6 mmol) and H_2SO_4 (0.07 mL) were added at room temperature. After stirring for 12 h at the same temperature, the volatile solvent was removed in vacuo to leave a residue. The residue was treated with H_2O (30 mL) and extracted twice with $CHCl_3$. The extract was washed with H_2O, saturated $NaHCO_3$ aqueous solution, and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give crude 2,3,4-trimethoxyphenol (1.1 g), which was used for the next step without further purification.

^1H NMR ($CDCl_3$; 400 MHz) δ 6.63 (1H, d, $J = 9.0$ Hz), 6.56 (1H, d, $J = 9.0$ Hz), 5.39 (1H, s), 3.96 (3H, s), 3.90 (3H, s), 3.81 (3H, s).

2,3,4-Trimethoxyphenyl acetate

To the crude 2,3,4-trimethoxyphenol (1.1 g) and pyridine (2.0 g, 25.5 mmol) in $CHCl_3$ (20 mL), Ac_2O (1.9 mL, 20.4 mmol) and pyridine (12 mL, 95.4 mmol) were added at room temperature under Ar gas. After stirring for 6 h at the same temperature, the reaction mixture was treated with H_2O and extracted with ethyl acetate (EtOAc). The extract was washed with saturated $KHSO_4$ aqueous solution, brine, saturated $NaHCO_3$ aqueous solution, and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give 2,3,4-trimethoxyphenylacetate (1.2 g), which was used for the next step without further purification. ^1H NMR ($CDCl_3$; 400 MHz) δ 6.71 (1H, d, $J = 9.0$ Hz), 6.59 (1H, d, $J = 9.0$ Hz), 3.86 (3H, s), 3.85 (3H, s), 3.82 (3H, s), 2.28 (3H, s).

1-(2-Hydroxy-3,4,5-trimethoxyphenyl)ethanone (4)

To the crude 2,3,4-trimethoxyphenylacetate (1.2 g), $BF_3{\cdot}OEt_2$ (4 mL, 31.8 mmol) was added at room temperature under Ar gas. After stirring for 30 min at 100 °C, the reaction mixture was poured into H_2O, and the resulting mixture was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ aqueous solution, brine, and then dried over Na_2SO_4. Removal of the solvent gave a

residue, which was purified by column chromatography using hexane-EtOAc (8:2, v/v) as the eluent to give 1-(2-hydroxy-3,4,5-trimethoxyphenyl)ethanone (**4**) (690.1 mg, 68%) as a yellow solid. ^{1}H-NMR ($CDCl_3$; 400 MHz) δ 12.44 (1H, s), 6.93 (1H, s), 4.04 (3H, s), 3.92 (3H, s), 3.86 (3H, s), 2.59 (3H, s); ^{13}C-NMR ($CDCl_3$; 100 MHz) δ 203.1, 153.1, 149.9, 145.0, 141.4, 114.2, 107.4, 61.3, 61.0, 59.7, 26.7.

1-(2-[Benzyloxy]-3,4,5-trimethoxyphenyl)ethanone (5)

To a solution of 1-(2-hydroxy-3,4,5-trimethoxyphenyl) ethanone (**4**) (94.6 mg, 0.42 mmol) in acetone, K_2CO_3 (122.0 mg, 0.88 mmol) and BnBr (74.6 µL, 0.63 mmol) were added at room temperature under Ar gas. After stirring for 49.5 h at the same temperature, the reaction mixture was diluted with EtOAc and then filtered through a pad of Celite. The filtrate was removed in vacuo. The residue was purified by column chromatography on silica gel using hexane-EtOAc (8:2, v/v) as the eluent to give benzyl ether **5** (124.9 mg, 94%) as a yellowish oil. IR ν_{max} 1672, 1117, 1065 cm^{-1}; ^{1}H NMR ($CDCl_3$; 400 MHz) δ 7.45–7.33 (5H, m), 5.06 (1H, s), 3.99 (3H, s), 3.94 (3H, s), 3.87 (3H, s), 2.56 (3H, s); ^{13}C NMR ($CDCl_3$; 100 MHz) δ 198.5, 149.3, 147.1, 147.1, 147.0, 136.8, 128.6, 128.3, 128.3, 127.5, 106.5, 76.4, 61.4, 61.2, 56.1, 31.4; HRMS (ESI^+) calcd for $C_{18}H_{21}O_5$ [M+H]: 317.1389; found: 317.1392.

Methyl 2-(2-[benzyloxy]-3,4,5-trimethoxyphenyl)acetate (6)

To a solution of the benzyl ether **5** (54.1 mg, 0.17 mmol) in MeOH (2 mL), H_2SO_4 (8.4 mg, 0.09 mmol) and trimethyl orthoformate (0.6 mL) were added at room temperature. After stirring for 10 min at the same temperature, HMBI (**7**, 1*H*-1-hydroxy-5-methyl-1,2,3-benziodoxanthiole 3,3-dioxide) (54 mg, 0.17 mmol) was added to the reaction mixture. After stirring for 15 min, HMBI (60 mg, 0.19 mmol) was added to the above mixture. After stirring for 2 h, the reaction mixture was poured into H_2O, and the resulting mixture was extracted with EtOAc. The extract was washed with saturated $NaHCO_3$ aqueous solution, brine, and dried over Na_2SO_4. Removal of the solvent gave a residue, which was purified by column chromatography on silica gel using hexane-EtOAc (8:2, v/v) as the eluent to give phenylacetate **6** (38.8 mg, 66%) as a yellowish oil. IR ν_{max} 1739, 1494, 1464, 1128, 1087 cm^{-1}; ^{1}H NMR ($CDCl_3$; 400 MHz) δ 7.49–7.30 (5H, m), 6.52 (1H, s), 4.99 (2H, s), 3.94 (3H, s), 3.91 (3H, s), 3.83 (3H, s), 3.64 (3H, s), 3.56 (2H, s); ^{13}C NMR ($CDCl_3$; 100 MHz) δ 172.2, 149.4, 147.1, 144.3, 142.3, 137.6, 128.3, 128.1, 127.9, 122.4, 108.2, 75.1, 61.1, 56.1, 51.9, 35.4; HRMS (ESI^+) calcd for $C_{19}H_{22}NaO_6$ [M+Na]: 369.1314; found: 369.1322.

Methyl 2-(2-[benzyloxy]-6-bromo-3,4,5-trimethoxyphenyl) acetate (8)

To a solution of methyl phenylacetate **6** (77.4 mg, 0.33 mmol) in MeOH-H_2O (1:1, 6 mL), NH_4Br (33.9 mg, 0.35 mmol) and oxone (151.3 mg, 0.25 mmol) were added at 0 °C. After stirring for 30 min at the same temperature, the reaction was quenched with H_2O, and the whole sample was extracted with EtOAc. The extract was washed with saturated $Na_2S_2O_3$ aqueous solution, saturated $NaHCO_3$ aqueous solution, and brine, and then dried over Na_2SO_4. The solvent was removed in vacuo to leave a residue, which was purified by column chromatography on silica gel using hexane-EtOAc (85:15, v/v) as the eluent to give bromobenzene **8** (64.1 mg, 67%) as a colorless oil. IR ν_{max} 1743, 1415, 1083, 1042 cm^{-1}; ^{1}H NMR ($CDCl_3$; 400 MHz) δ 7.43–7.32 (5H, m), 5.00 (2H, s), 3.97 (3H, s), 3.93 (3H, s), 3.87 (3H, s), 3.82 (2H, s), 3.66 (3H, s); ^{13}C NMR ($CDCl_3$; 100 MHz) δ 171.3, 147.8, 147.4, 147.1, 146.5, 137.2, 128.5, 128.1, 128.1, 124.2, 114.8, 75.6, 61.4, 61.2, 61.0, 52.0, 35.7; HRMS (ESI^+) calcd for $C_{19}H_{21}{}^{79}BrNaO_6$ [M+Na]: 447.0419; found: 447.0406.

Methyl 2-(2-bromo-6-hydroxy-3,4,5-trimethoxyphenyl) acetate (9)

To a solution of bromobenzene **8** (52.5 mg, 0.12 mmol) in MeOH (4 mL), 10% Pd/C (4.2 mg) was added under an atmosphere of H_2 at room temperature. After stirring for 1.5 h at the same temperature, the reaction mixture was filtered through a pad of celite. The filtrate was concentrated to leave a residue, which was used for the next steps without further purification.

Methyl 2-(2-bromo-4,5-dimethoxy-3,6-dioxocyclohexa-1,4-dienyl)acetate (10)

To a solution of the corresponding phenol **9** (45.4 mg) in CH_3CN-H_2O (2:1, 6 mL), $n$$Bu_4NBr$ (4.7 mg, 0.01 mmol) and oxone (64.7 mg, 0.11 mmol) were added at 0 °C. After stirring for 2.5 h at the same temperature, the reaction mixture was filtered through a pad of SiO_2. The filtrate was concentrated in vacuo to leave a residue, which was purified by column chromatography on silica gel using hexane-EtOAc (30%, v/v) as the eluent to give quinone **10** (30.1 mg, 76%) as a reddish oil. IR ν_{max} 1743, 1669, 1604, 1262 cm^{-1}; ^{1}H NMR ($CDCl_3$; 400 MHz) δ 4.05 (3H, s), 4.03 (3H, s), 3.76 (2H, s), 3.73 (3H, s); ^{13}C NMR ($CDCl_3$; 100 MHz) δ 180.1, 176.3, 168.4, 145.1, 144.3, 139.9, 136.0, 61.6, 61.3, 52.5, 35.8; HRMS (ESI^+) calcd for $C_{11}H_{12}{}^{79}BrO_6$ [M+H]: 318.9817; found: 318.9808.

Coptirhoquinone A (1)

To a solution of quinone **10** (20.6 mg, 0.06 mmol) in $CHCl_3$-H_2O (6:1, 3.5 mL), $n$$Bu_4NBr$ (3 mg, 9.3 μmol) and NaSMe (16.9 mg, 0.24 mmol) were added at room temperature. After stirring for 20 min at the same temperature, the reaction mixture was filtered through a pad of SiO_2. The filtrate was concentrated in vacuo to leave a residue, which was purified by column chromatography using hexane-EtOAc (4:1, v/v) as the eluent to give thioquinone **1** (14.1 mg, 76%) as a reddish oil. ^{1}H NMR ($CDCl_3$; 400 MHz) δ 4.02 (3H, s), 4.01 (3H, s), 3.78 (2H, s), 3.71 (3H, s), 2.57 (3H, s); ^{13}C NMR ($CDCl_3$; 100 MHz) δ 180.9, 180.0, 169.5, 145.4, 145.2, 144.7, 137.7, 61.3, 61.2, 52.3, 33.5, 17.7.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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