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Corsifurans A–C, 2-arylbenzofurans of presumed stilbenoid origin from *Corsinia coriandrina* (Hepaticae)

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Abstract

Chemical investigation of the diethyl ether extract from the liverwort *Corsinia coriandrina* resulted in the isolation of a new 2-arylbenzofuran compound called corsifuran A. The structure was identified by spectroscopic techniques and confirmed by synthesis. Two minor constituents of similar structure, and two related stilbenoids and a bibenzyl were identified by comparison of the mass spectra and GC retention indices with authentic samples. Due to the similarity in substitution patterns a stilbenoid origin of the corsifurans is proposed.

Keywords: *Corsinia coriandrina*; Liverworts; 2-Arylbenzofurans; Stilbenoids; Structure elucidation; Synthesis

1. Introduction

Several 2-arylbenzofurans originating from the phenylpropanoid-polymalonate pathway have previously been described, mainly from the Fabaceae (Leguminosae) and Moraceae. A considerable number is known to occur as phytoalexins, specific stress metabolites produced de novo in response to microbial or fungal infections as well as to abiotic factors. These compounds exhibit antifungal (Carter et al., 1978; Nomura, 1988), antibacterial (Demizu et al., 1988; Tanaka et al., 2003; Erasto et al., 2004), insect feeding deterrent (Russell et al., 1984), free radical scavenging (Sharma et al., 2001; Erasto et al., 2004), antioxidant (Demizu et al., 1988), cyclooxygenase inhibiting (Su et al., 2002), and estrogenic activities (Halabalaki et al., 2000; Collini et al., 2004).

Comparison of the substitution patterns and labelling experiments have revealed isoflavonoid or stilbenoid pathways for 2-arylbenzofuran biogenesis (Fig. 1). The 2′,4′,6-trioxygenated 2-arylbenzofurans (**4**) of the Fabaceae are considered to be of isoflavonoid origin and derive from loss of one carbon unit during ring contraction of a coumestan (**3**) as shown for the phytoalexin vignafuran from *Vigna unguiculata* (Preston et al., 1975; Martin and Dewick, 1979; Kinoshita, 1997). For the moracin phytoalexins and related antifungal compounds with a 3′,5′,6-trioxygenated 2-arylbenzofuran structure (**7**) obtained from mulberry (*Morus* sp.) a stilbenoid origin has been considered (Nomura, 1988) due to the co-occurrence of structurally similar resveratrol (**5**) and oxyresveratrol (**6**) (Takasugi et al., 1978). Closely related stilbenoids and 2-arylbenzofurans as well as the corresponding glucosides have also been described from *Schoenocaulon officinale* (Liliaceae) (Kanchanapoom et al., 2002).

In comparison to flavonoids (**2**), isoflavonoids (**3**) and stilbenoids (**8**) are very rare in bryophytes, but bibenzyls and bisbibenzyls are widely distributed among the liverworts (Asakawa, 1995, 2004). Nevertheless, licarin A from *Jackiella javanica* (Nagashima et al., 1990) and egonol 2-methylbutanoate from *Riccardia multifida*

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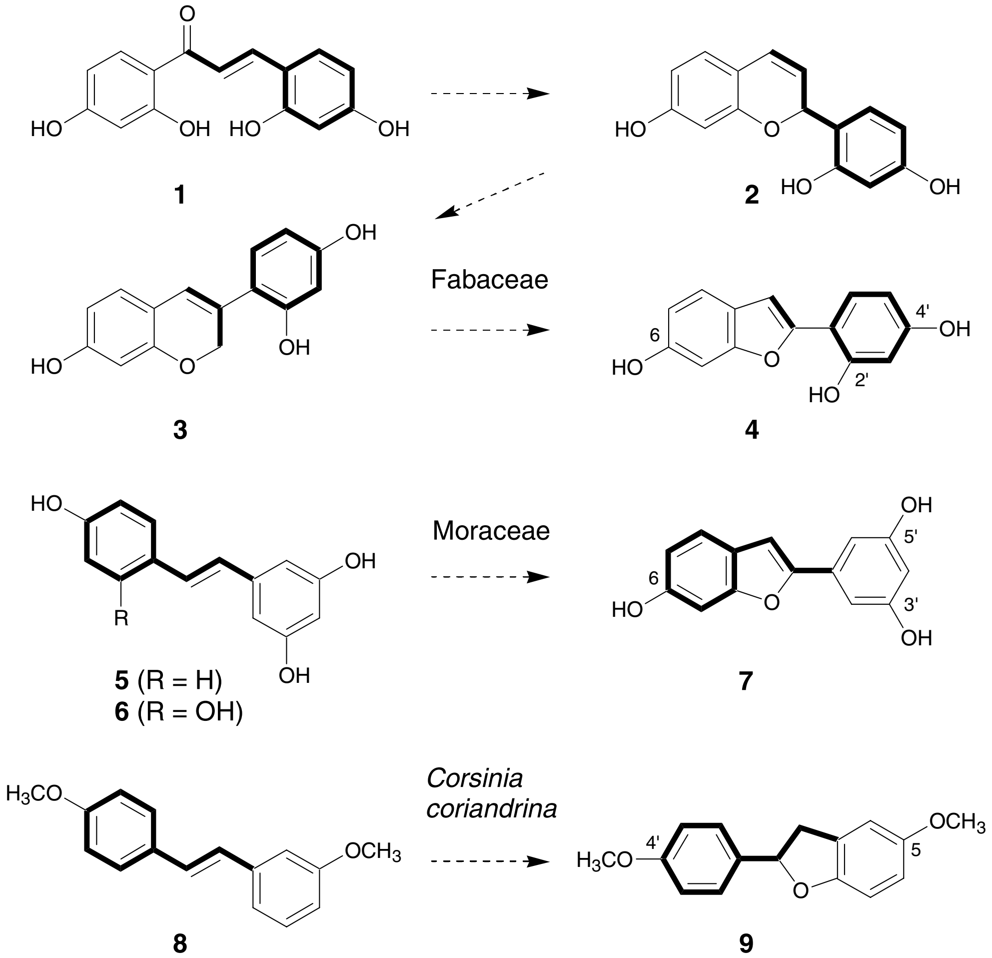

Fig. 1. Biogenetic pathways for 2-arylbenzofurans of phenylpropanoid–polymalonate origin (phenylpropanoid unit drawn bold).

subsp. *decrescens* (Yoshida et al., 1997), which represent the only 2-arylbenzofuran compounds reported from the bryophytes so far, are apparently of neolignan (bisarylpropenoid) origin.

We now like to report on the isolation, structure elucidation and synthesis of corsifuran A (**9**) with a 4′,5-dioxygenated 2-arylbenzofuran skeleton of presumed stilbenoid origin from the Mediterranean liverwort *Corsinia coriandrina*.

2. Results and discussion

From the diethyl ether extract of *Corsinia coriandrina* (Spreng.) Lind. (Corsiniaceae), collected in Andalusia, Spain, one unidentified major component (**10**, 7.1% of the total volatiles) was isolated by a combination of repeated column chromatography on SiO_2 using a *n*-hexane–diethyl ether gradient and a *n*-hexane–dichloromethane gradient, followed by preparative thin-layer chromatography using a *n*-hexane–ethyl acetate mixture. The mass spectrum exhibited a highly predominating molecular ion peak at $m/z = 256$ $[M]^+$ and several fragment ion signals of low intensity, with those at $m/z = 241$ $[M–CH_3]^+$, $m/z = 225$ $[M–OCH_3\ ]^+$ and $m/z = 213$ $[M–CH_3–CO]^+$ indicating a methoxy substituted aromatic structure. By HRMS, the molecular formula of $C_{16}H_{16}O_3$ with 9 degrees of unsaturation could be established. The ^1H NMR spectrum exhibited signals for one 1,4-disubstituted and one 1,2,4-trisubstituted benzene unit, two methoxy groups and one anisochoric methylene group adjacent to a stereogenic methine group (Table 1). Inspection of the H,H-COSY spectrum revealed a bibenzyl skeleton, whereas the chemical shift of the stereogenic methine group (δ_H 5.48) and the anisochrony of the adjacent methylene group (δ_H 2.90 and 3.09) indicated a 2-(4-methoxyphenyl)-2,3-dihydrobenzofuran structure with a 5- (or 6-) methoxy substitution, which we name corsifuran A (**10**).

Two additional minor constituents with similar fragmentation patterns, called corsifuran B (**11**, 1.5%) ($C_{15}H_{14}O_3$, $m/z = 242.0961$) and corsifuran C (**12**, 1.1%) ($C_{16}\ H_{14}O_3$, $m/z = 254.0965$), were tentatively

Table 1
^{1}H NMR data for corsifuran A–C in C_6D_6 (500 MHz, δ_H in ppm, integral, multiplicity, *J* in Hz; assignments made by ^{1}H NMR and COSY experiments)

^{1}H	Corsifuran A (**10**)	Corsifuran B (**11**)	Corsifuran C (**12**)
2	5.48 1H *t* 8.6	5.44 1H *t* 8.8	–
3	2.90 1H *dd* 15.4, 8.5	2.84 1H *dd* 15.8, 8.5	6.61 1H *d* 0.8
3′	3.09 1H *dd* 15.8, 9.1	3.02 1H *dd* 15.8, 9.1	–
4	6.70 1H *d* 2.8	6.45 1H *d* 2.5	6.96 1H *d* 2.5
5-OCH_3	3.38 3H *s*	–	3.44 3H *s*
6	6.62 1H *dd* 8.5, 2.8	6.33 1H *dd* 8.6, 2.5	6.84 1H *dd* 8.9, 2.5
7	6.83 1H *d* 8.5	6.73 1H *d* 8.8	7.30 1H *d* 8.9
2′6′	7.20 2H *d* 8.8	7.17 2H *d* 8.8	7.75 2H *d* 8.9
3′5′	6.75 2H *d* 8.8	6.74 2H *d* 8.8	6.79 2H *d* 8.9
4′-OCH_3	3.28 3H *s*	3.27 3H *s*	3.27 3H *s*

identified as the corresponding *O*-desmethyl and 2,3-dehydro derivatives, respectively.

The 2-(4-methoxyphenyl)-benzofuran skeleton of the corsifurans and the 5-*O*-substitution were proven unambiguously by synthesis as shown in Fig. 3. Although a synthetic access to corsifuran A (**10**) (Gates et al., 1992) and corsifuran C (**12**) (Thomas and Bokadia, 1968; Guy and Guette, 1980) has already been described, we followed a modification of an alternative literature procedure (Ohara et al., 2002). Cycloaddition between 4-methoxystyrene (**18**) and *p*-quinone (**20**) promoted by ferric(III)chloride hexahydrate in acetonitrile afforded 5-hydroxy-2-(4-methoxyphenyl)-2,3-dihydrobenzofuran (**11**) in moderate yield, which was shown to be identical to corsifuran B by comparison of the mass spectra and gas chromatographic retention indices on different stationary phases. Methylation of **11** afforded corsifuran A (**10**), which exhibited MS and ^{1}H NMR data identical to the natural product isolated from *C. coriandrina*. Dehydrogenation of **10** using 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in dioxane afforded corsifuran C (**12**) as shown by GC–MS.

In addition, one unidentified minor constituent with a highly dominating signal for the molecular ion at $m/z = 240$ was detected in the diethyl ether extract of *Corsinia coriandrina* (**13**, 1.3% of the total volatiles). The mass spectrometric fragmentation pattern indicated a methoxy substituted aromatic compound. HRMS afforded the molecular formula $C_{16}H_{16}O_2$ with 9 degrees of unsaturation and, thus, suggested a dimethoxystilbene structure. By comparison with an authentic sample prepared according to modified literature procedures (*E*)-3,4′-dimethoxystilbene (**13**) could be identified in a liverwort for the first time (Huneck, 1976). Traces of the isomeric (*Z*)-3,4′-dimethoxystilbene (**14**, 0.2%) and 3,4′-dimethoxybibenzyl (**15**, 0.1%) were also detected by GC–MS (Fig. 2).

Although corsifuran A and C (**10**, **12**) have previously been described as synthetic compounds this is the first report on their identification as natural products. The unusual 4′,5-*O*-disubstitution of the corsifurans (**10**–**12**) has so far only been described for the corresponding

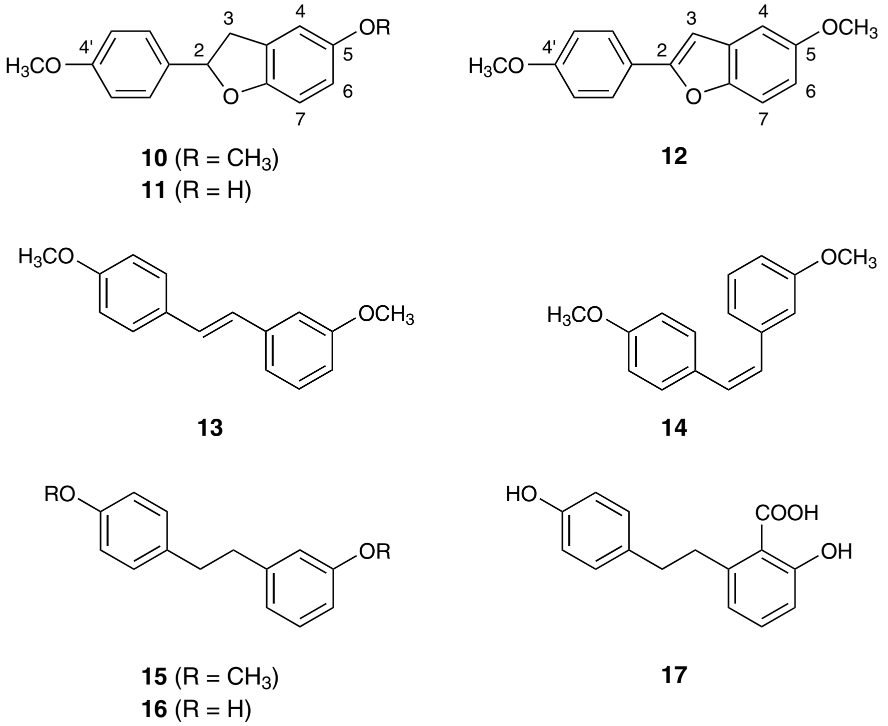

Fig. 2. 2-Arylbenzofurans (**10**–**12**), stilbenoids (**13**, **14**) and bibenzyls (**15**–**17**) from *Corsinia coriandrina*.

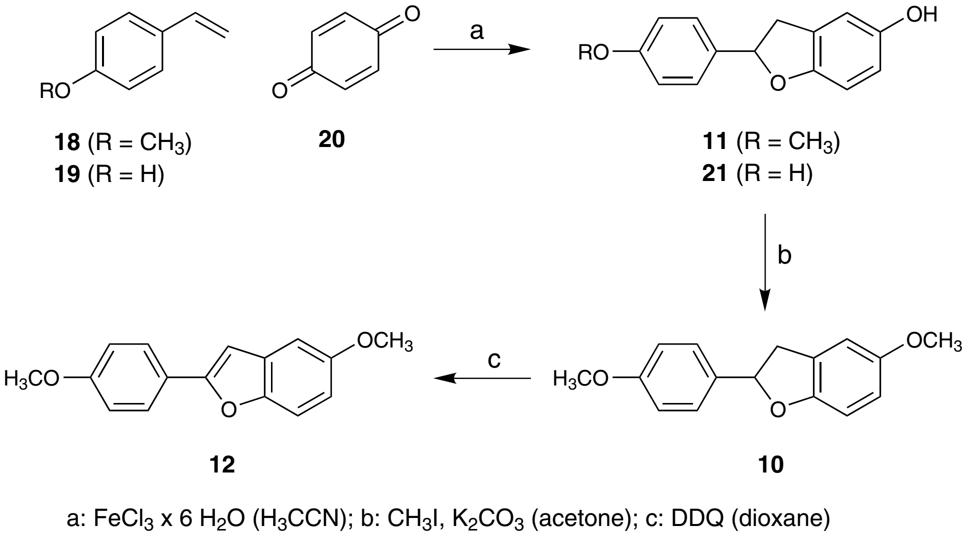

Fig. 3. Synthesis of corsifuran A–C (**10**–**12**) and previously proposed metabolic pathway of *p*-coumaric acid in *Bacillus megaterium* via **19** to **21**.

O,O-desmethyl derivative (**21**), known as a racemic metabolite of exogenic *p*-coumaric acid in *Bacillus megaterium* (Torres y Torres and Rosazza, 2001). Due to the co-occurrence of 4-hydroxystyrene (**19**), a biogenetic pathway similar to that of corsifuran B (**11**) was suggested (Fig. 3), although the origin of the required *p*-quinone (**20**) remained questionable. In contrast to racemic **21**, high enantiopurity of corsifuran A (**10**) could be established by enantioselctive GC using an octakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin column (König, 1987).

The similarity in substitution patterns between corsifuran A–C (**10**, **11**, **12**), (*E*)- and (*Z*)-3,4′-dimethoxystilbene (**13**, **14**) and 3,4′-dimethoxybibenzyl (**15**) with the liverwort-specific dormancy inducing factor lunularic acid (**17**) and its decarboxylation product lunularin (**16**) (Fig. 2), previously isolated from *Corsinia coriandrina* (Gorham, 1977), indicates a common phenylpropanoid–polymalonate pathway (Eckermann et al., 2003). Furthermore, the co-occurrence of 3,4′-dimethoxystilbenes (**8**) suggests a stilbenoid origin for the corsifurans (**9**) as shown in Fig. 1. Hence, the A ring of the corsifurans (**9**) should originate from the phenylpropanoid unit in contrast to the oxyresveratrol derived moracins (**7**) obtained from Moraceae (Fig. 1). Biological tests with the synthetic compounds and investigation of their absolute stereochemistry and biogenesis are in progress.

3. Experimental

3.1. General experimental procedures

3.1.1. GC, GC–MS and GC–HRMS

Gas chromatograms were run using a Carlo Erba HRGC 5300 Mega instrument equipped with 25 m fused silica capillary columns with polysiloxanes CPSil-5 and CPSil-19 (Chrompack) or Carlo Erba Fractovap 4160 instrument equipped with a 25 m fused silica capillary column with octakis-(2,6-di-*O*-methyl-3-*O*-pentyl)-γ-cyclodextrin in OV-1701 (1:1; w/w). Hydrogen at 0.5 bar inlet pressure was used as carrier gas. Split injection at 200 °C (split ratio: 1:30); flame ionisation detector at 250 °C. Temp. progr. 50–250 °C at 3 °C/min. Electron impact (70 eV) GC–MS and GC–HRMS measurements were carried out on a Hewlett Packard HP 5890 gas chromatograph equipped with a 25 m CPSil-5 CB (Chrompack) fused-silica capillary column coupled to a VG Analytical 70-250S mass spectrometer.

3.1.2. NMR spectroscopy

NMR measurements were carried out with a Bruker WM 400 instrument for broadband decoupled ^{13}C NMR and ^{13}C Pendant spectra (100.6 MHz) and with WM 500 instrument for ^{1}H NMR, H,H-COSY, HMQC and HMBC spectra (^{1}H: 500.1 MHz, ^{13}C: 125.8 MHz). Benzene-d_6 was used as solvent and TMS served as internal standard.

3.2. Plant material and extracts

Plant material of *Corsinia coriandrina* (Spreng.) Lind. was collected near Tarifa and near St. Bartolomeo, Andalusia, Spain in March 2003 and April 2004, respectively. The liverworts were identified by Professor R. Mues, (Universität des Saarlands, Germany) and Dr. H. Muhle (Universität Ulm, Germany). After careful cleaning the air dried plant material (10 g) was powdered under liquid nitrogen and extracted with diethyl ether.

3.3. Isolation of corsifuran A (10)

The crude diethyl ether extract was chromatographed on silica gel 60 F_{254} (Merck) using a *n*-hexane–diethyl

Table 2
^{13}C NMR data for corsifuran A–C in C_6D_6 (100 MHz, δ_C in ppm, multiplicity; assignments made by PENDANT, HMQC and HMBC experiments)

^{13}C	Corsifuran A (**10**)	Corsifuran B (**11**)	Corsifuran C (**12**)
2	84.3 *d*	84.3 *d*	157.6 *s*
3	39.1 *t*	38.9 *t*	100.7 *d*
3a	128.3 *s*	128.3 *s*	130.9 *s*
4	111.7 *d*	112.5 *d*	104.0 *d*
5	154.9 *s*	150.6 *s*	157.1 *s*
5-OCH_3	55.6 *q*	–	55.1 *q*
6	113.2 *d*	114.6 *d*	113.1 *d*
7	109.5 *d*	109.5 *d*	112.0 *d*
7a	154.6 *s*	154.3 *s*	150.6 *s*
1′	134.8 *s*	134.7 *s*	124.2 *s*
2′6′	127.5 *d*	127.5 *d*	127.0 *d*
3′5′	114.2 *d*	114.2 *d*	114.9 *d*
4′	159.9 *s*	159.9 *s*	160.8 *s*
4′-OCH_3	54.8 *q*	54.8 *q*	55.7 *q*

ether gradient and a *n*-hexane–dichloromethane gradient, respectively. Preparative thin-layer chromatography on glass plates with silica gel 60 F_{254} (Merck) using a *n*-hexane–ethyl acetate mixture (10/1, $R_f = 0.17$, UV detection) afforded 0.5 mg corsifuran A (0.005% of the dry weight).

5-Methoxy-2-(4-methoxyphenyl)-2,3-dihydrobenzofuran. White solid; ^1H and ^{13}C NMR data: see Tables 1 and 2, respectively; MS and HRMS data: see Section 3.5.

3.4. Synthesis of corsifuran B (11)

A solution of 270 mg *p*-quinone **20** (2.5 mmol) in 10 ml acetonitrile was treated with 13.5 mg $FeCl_3$ hexahydrate (0.05 mmol), and a solution of 335 mg 4-methoxystyrene **18** (2.5 mmol) in 1 ml acetonitrile was added dropwise. After stirring at room temperature for 6 h the reaction mixture was poured into 15 ml water and extracted with 2 × 15 ml dichloromethane. The organic phase was washed with 2 × 25 ml water, dried over sodium sulphate and concentrated. Column chromatography on silica (elution with dichloromethane) afforded 230 mg 5-hydroxy-2-(4-methoxyphenyl)-2,3-dihydrobenzofuran (0.95 mmol, 38% yield) identical to the natural product (**11**) from *C. coriandrina*.

5-Hydroxy-2-(4-methoxyphenyl)-2,3-dihydrobenzofuran. White solid; ^1H and ^{13}C NMR data: see Tables 1 and 2, respectively; MS (EI, 70 eV): *m/z* (%): 242 (100) $[M]^+$, 227 (6), 199 (5), 181 (16), 165 (2), 153 (7), 141 (2), 134 (8), 128 (6), 121 (4), 115 (15), 91 (6), 77 (11), 65 (9), 55 (18), 51 (10), 39 (10); HRMS $m/z = 242.0961$ $[M]^+$ (calc. for $C_{15}H_{14}O_3$: 242.0943).

3.5. Synthesis of corsifuran A (10)

A solution of 140 mg **11** (0.58 mmol) in 3 ml acetone was treated with 0.5 g potassium carbonate and 170 mg methyl iodide (1.2 mmol). After 12 h, the mixture was filtered, concentrated and chromatographed on silica using a *n*-hexane–ethyl acetate mixture (10/1) to give 140 mg 5-methoxy-2-(4-methoxyphenyl)-2,3-dihydrobenzofuran (0.55 mmol, 95% yield) identical to the natural product (**10**) isolated from *C. coriandrina*.

5-Methoxy-2-(4-methoxyphenyl)-2,3-dihydrobenzofuran. White solid; ^1H and ^{13}C NMR data: see Tables 1 and 2, respectively; MS (EI, 70 eV): *m/z* (%): 256 (100) $[M]^+$, 241 (6), 225 (2), 213 (3), 198 (4), 181 (4), 171 (2), 165 (2), 148 (6), 141 (3), 128 (4), 115 (6), 91 (3), 77 (5), 55 (8), 51 (6); HRMS $m/z = 256.1091$ $[M]^+$ (calc. for $C_{16}H_{16}O_3$: 256.1099).

3.6. Synthesis of corsifuran C (12)

A solution of 51 mg **10** (0.2 mmol) in 1 ml dioxane was treated with 48 mg 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (0.21 mmol) and heated to 100 °C for 14 h. Crystals formed after cooling were washed with *n*-hexane and the combined solution concentrated in vacuum. The residue was chromatographed on silica (elution with dichloromethane) to give 48 mg 5-methoxy-2-(4-methoxyphenyl)-benzofuran (0.19 mmol, 95% yield) identical to the natural product (**12**) from *C. coriandrina*.

5-Methoxy-2-(4-methoxyphenyl)-benzofuran. Off-white solid; ^1H and ^{13}C NMR data: see Tables 1 and 2, respectively; MS (EI, 70 eV): *m/z* (%): 254 (100) $[M]^+$, 239 (44), 223 (2), 211 (18), 196 (5), 183 (7), 168 (20), 152 (10), 139 (16), 127 (7), 114 (6), 63 (7); HRMS $m/z = 254.0965$ $[M]^+$ (calc. for $C_{16}H_{14}O_3$: 254.0943).

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