

Antifungal 2-(2-Hydroxyphenyl)benzofuran Identified in Guaiacol Pyrolyzate

Kazuo Narita and Haruo Kawamoto*

Graduate School of Energy Science, Kyoto University, Kyoto 606-8501, Japan

*Corresponding author: kawamoto.haruo.2m@kyoto-u.ac.jp

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Abstract

2-(2-Hydroxyphenyl)benzofuran, a previously unreported product of guaiacol pyrolysis, was isolated from guaiacol pyrolyzed at 600 °C in a yield of 1.5 wt%. A plausible formation pathway is proposed, involving the addition of *o*-cresol—formed during guaiacol pyrolysis—to an *o*-quinone methide intermediate generated via methoxy group rearrangement. This compound is known for its strong antifungal activity, suggesting it may contribute to the antifungal properties of tar and pyroligneous acids derived from the pyrolysis of lignin and wood.

Keywords: Antifungal activity; Phenylbenzofuran derivative; Pyrolysis; *o*-Quinone methide

INTRODUCTION

Wood tar and pyroligneous acid, derived from wood pyrolysis, have long been recognized for their diverse biological activities and have been utilized in various applications^{1–6}. In pharmaceuticals, creosote, primarily composed of wood tar constituents, has a well-documented history as a medicinal agent^{4,5,7–11}. In the food industry, smoking—using wood smoke generated by incomplete combustion—has been practiced worldwide to enhance food preservation and flavor^{3,12}. Pyroligneous acid has also found applications in Asia, including Japan, as an insecticide, repellent, and agricultural input^{1,2}, with growing interest in its use as an eco-friendly alternative to synthetic pesticides^{1,2,6}. Among their biological properties, antimicrobial and antifungal activities have received significant attention.

Most studies^{6,13–18} have focused on pyroligneous acid and reported its antifungal activity against brown-rot fungi (*Fomitopsis palustris*, *Gloeophyllum trabeum*), white-rot fungi (*Trametes versicolor*, *Rigidoporus amylospora*), sap-stain fungi (*Ophiostoma flexuosum*), and plant pathogens (*Alternaria mali*, *Botryodiplodia theobromae*). Oramahi et al.¹⁶ found that pyroligneous acid produced at 450 °C exhibits the highest antifungal activity among samples carbonized at 350, 400, and 450 °C. While organic acids and phenolic compounds in pyroligneous acid are believed to contribute to this activity, detailed studies using isolated compounds are scarce. Velmurugan et al.¹⁵ demonstrated that antifungal activity persists even after neutralization of pyroligneous acid.

Lignin pyrolysis studies have revealed that decomposition at 300–400 °C primarily affects the propyl side chains, resulting in cleavage of ether linkages such as the β -ether bond¹⁹. At approximately 450 °C, homolytic cleavage of O–CH₃ bonds in methoxy groups becomes dominant, leading to methoxy-group rearrangement. In guaiacyl units, these higher-temperature reactions result in the formation of catechols, *o*-cresols, xanthenes, polycyclic aromatic hydrocarbons (PAHs), and coke¹⁹. During guaiacol pyrolysis at 600 °C, we isolated and identified 2-(2-hydroxyphenyl)benzofuran, a compound not previously reported from lignin pyrolysis and known for its strong antifungal activity^{20,21}.

RESULTS AND DISCUSSION

Guaiacol (10 mg) was sealed in a nitrogen-purged Pyrex ampoule and pyrolyzed at 600 °C for 120 s. The methanol extract of the pyrolyzate was analyzed by gas chromatography–mass spectrometry (GC/MS) (Fig. 1). Major signals corresponded to catechol (**1**), *o*-cresol (**2**), and phenol (**3**), formed via O–CH₃ bond homolysis and transformation. Additional signals included *o*-ethylphenol (**4**), benzofuran (**5**), and xanthenes (**6** to **8**). A prominent peak for unknown compound A was observed at 32.5 min.

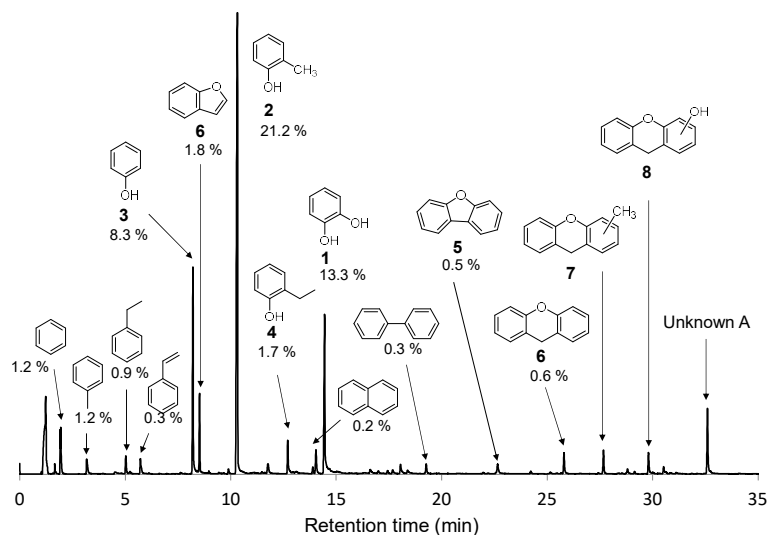


Fig. 1. Total ion chromatogram obtained from GC/MS analysis of the methanol-soluble pyrolyzates produced during the pyrolysis of guaiacol at 600 °C for 120 s in an ampoule reactor.

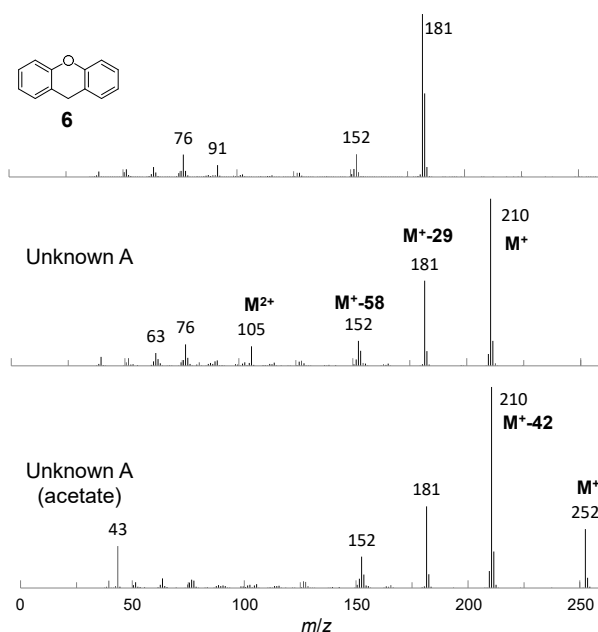


Fig. 2. Mass spectra of unknown compound A and its acetate derivative, compared with that of xanthene (**6**).



Thin-layer chromatography (TLC) separation of the methanol-soluble portion (EtOAc/hexane = 1:4, v/v) yielded five fractions: Fraction 1 (R_f = 0.20) contained catechol; Fraction 2 (R_f = 0.41), unknown A; Fraction 3 (R_f = 0.45), phenol; Fraction 4 (R_f = 0.54), *o*-cresol; and Fraction 5 (R_f = 0.88), xanthene derivatives. Further purification of Fraction 2 by TLC (chloroform) yielded unknown A as colorless crystals in 1.5 wt% from guaiacol.

Comparison of the mass spectrum of unknown A with those of its acetate and xanthene (**6**) is presented in Fig. 2. The presence of fragment ions at m/z 181, 152, and 76, similar to those observed for xanthene, suggested that unknown A is a dimeric product derived from guaiacol. A strong signal at m/z 210, 29 units above the $M^+ - 1$ ion (m/z 181) of **6**, indicates an additional CHO unit. Loss of CHO from the molecular ion explains the m/z 181 fragment. Acetylation of unknown A increased the mass by 42 units, indicating the presence of one hydroxy group in unknown A. A fragment at m/z 105 (half the molecular ion) was also noted.

The proton nuclear magnetic resonance (^1H NMR) and proton–proton correlation spectroscopy (H-H COSY) spectra of unknown A are shown in Fig. 3. In the ^1H NMR spectrum, all signals appeared downfield of 6.8 ppm, consistent with conjugated C=C double bonds. The carbon-13 nuclear magnetic resonance (^{13}C NMR) spectrum (Fig. 3) confirmed 14 carbon atoms—one more than xanthene. Three signals below 150 ppm suggest carbons bonded to oxygen atoms. These data led to the identification of unknown A as 2-(2-hydroxyphenyl)benzofuran (**9**). The H-H COSY spectrum enabled assignment of all protons, including a long-range coupling between positions 3 and 4.

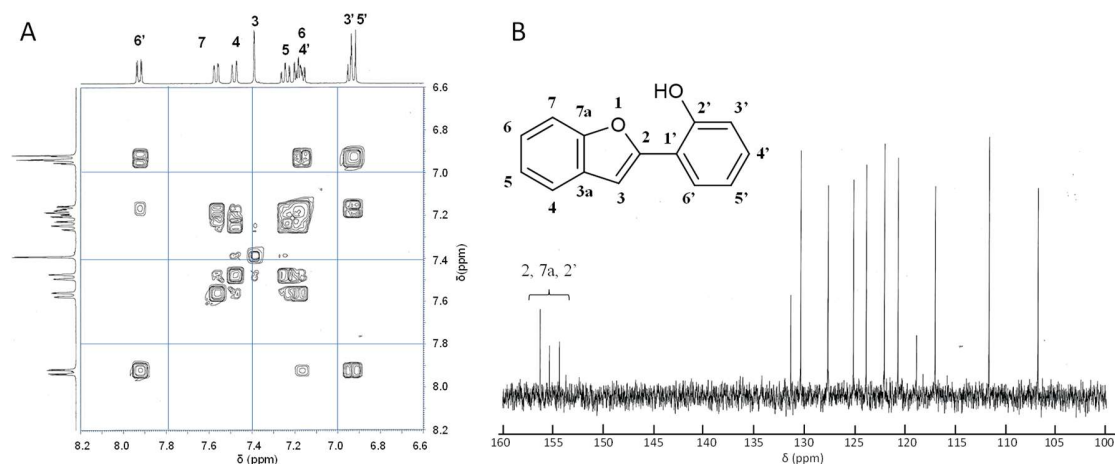


Fig. 3. H-H COSY (A) and ^{13}C NMR (B) spectra of isolated compound A, identified as 2-(2-hydroxyphenyl)benzofuran (**9**).

To confirm the proposed structure, compound **9** was synthesized according to the method of Bhatt et al.²²⁾ via Homo-McMurry coupling of salicylaldehyde (Zn/TiCl₄/THF/reflux) to afford stilbene, followed by cyclization using CuBr₂. The synthetic product matched the isolated compound in mass, ^1H NMR, H-H COSY, and ^{13}C NMR spectra, confirming the identity of unknown A as 2-(2-hydroxyphenyl)benzofuran (**9**).

The identification of unknown A as **9** also allowed the mass fragmentation pattern shown in Fig. 2 to be interpreted based on previous studies of 2-phenylbenzofurans²³⁾. These compounds characteristically form dications, a feature of heteroaromatic compounds with fragmentation-resistant bonds²³⁾. Thus, the ion at m/z 105, corresponding to half the molecular mass of **9**, was assigned to the molecular dication $[M]^{2+}$. The transition from m/z 210 to m/z 181 involves CHO loss from the phenol ring, whereas the subsequent m/z 181 to m/z 152 shift is due to CHO elimination from the heterocyclic ring²⁴⁾.

The proposed mechanism for 2-(2-hydroxyphenyl)benzofuran formation during guaiacol pyrolysis is shown in Fig. 4. A guaiacol-derived phenoxyl radical rearranges via methoxy migration to form *o*-quinone methide¹⁹⁾. Addition of a hydrogen radical followed by hydrogen-atom abstraction from

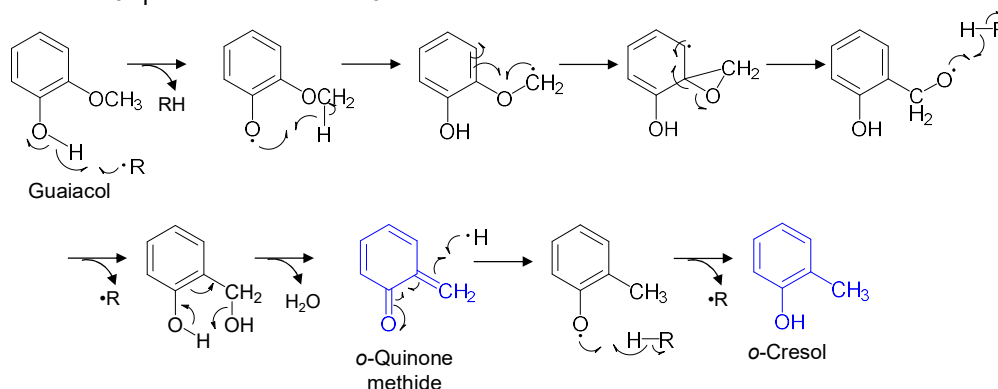


a hydrogen donor yields *o*-cresol. A benzyl radical from *o*-cresol adds to another *o*-quinone methide to form stilbene, which regenerates *o*-quinone methide. Intramolecular nucleophilic addition by a phenolic hydroxy group closes the heterocycle, followed by conjugated double bond formation via a benzyl radical.

2-Phenylbenzofuran derivatives have been isolated from various plants and are known for diverse biological activities, including antimicrobial, antifungal, antioxidant, anti-inflammatory, antitumor, and antiproliferative effects^{25–31}). Their pharmacological potential has driven extensive synthetic studies, and the synthesis of compound **9** has also been reported^{22,31–34}).

2-(2-Hydroxyphenyl)benzofuran has not been identified in wood tar or pyrolytic acid, so its contribution to their biological activities remains unexplored. However, its 1.5 wt% yield from guaiacol pyrolysis suggests that this compound or its analogs may be present and contribute to observed antimicrobial effects. Notably, previous studies^{20,21}) evaluating 56 synthetic 2-phenylbenzofuran derivatives including compound **9** for antifungal activity against phytopathogens (e.g., *Alternaria brassicicola*, *Botrytis cinerea*, *Septoria nodorum*, *Uromyces viciae-fabae*, *Cladosporium cucumerinum*, *Colletotrichum lagenarium*, *Ascochyta fabae*, *Glomerella cingulata*, *Fusarium culmorum*, *Aspergillus niger*) reported that compound **9**, bearing a 2'-hydroxy group, exhibited particularly strong antifungal activity.

Formation of *o*-quinone methide and *o*-cresol



Coupling of *o*-quinone methide with *o*-cresol, forming 2-(2-hydroxyphenyl)benzofuran **9**

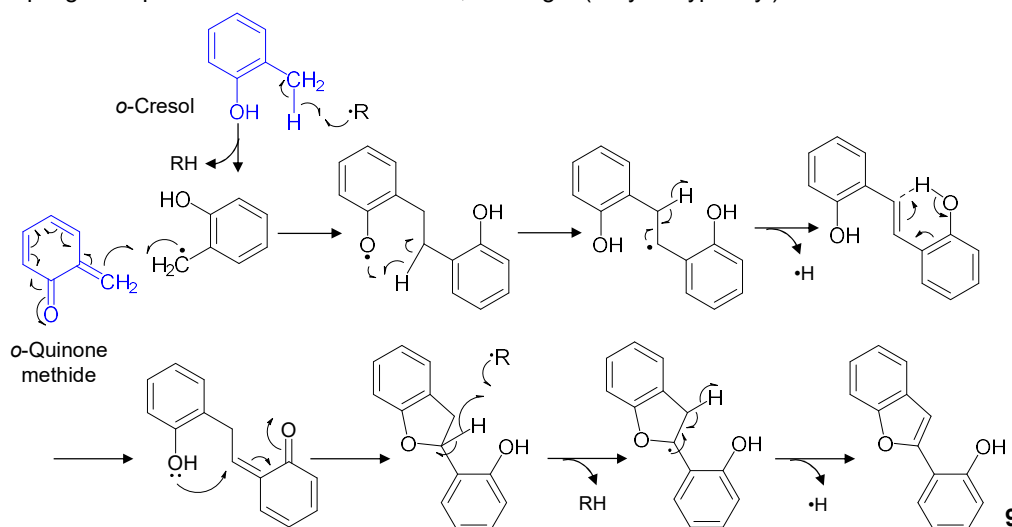


Fig. 4. Proposed formation mechanism of 2-(2-hydroxyphenyl)benzofuran (**9**) from guaiacol at 600 °C.



EXPERIMENTAL

Guaiacol, catechol, *o*-cresol, 2-ethylphenol, phenanthrene, and dibromobenzene were purchased from Nacalai Tesque, Inc. (Kyoto, Japan), while benzofuran, xanthene, and dibenzofuran were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). TLC was performed on silica gel plates (Kieselgel 60 F254, Merck KGaA, Darmstadt, Germany). NMR spectra were recorded on a Varian AC-400 (400 MHz) spectrometer (Varian, Palo Alto, CA, USA) using methanol-*d*₄ as the solvent. Tetramethylsilane (TMS) was used as the internal standard, and chemical shifts and coupling constants are reported in δ (ppm) and *J* (Hz), respectively.

Pyrolysis

Guaiacol (10 mg) was placed in a Pyrex glass ampoule (120 mm length, 8 mm inner diameter, 1 mm wall thickness) pre-washed with 1 N hydrochloric acid. The air inside the ampoule was replaced with nitrogen, and the ampoule was then sealed. It was then inserted into a muffle furnace preheated to 600 °C and heated for 120 s. After heating, the ampoule was sequentially cooled by air (1 min) and water (2 min) to quench the pyrolysis. The ampoule was opened, and the contents were extracted with methanol (1 mL) to obtain the methanol-soluble pyrolyzate.

GC/MS Analysis

The methanol extract was directly subjected to GC/MS. Unknown compound A and its acetate derivative, obtained by TLC separation, were also analyzed in methanol solution. Acetylation of unknown A was performed in acetic anhydride/pyridine.

GC/MS analysis was conducted using a GCMS-QP2010 Ultra system (Shimadzu Corp., Kyoto, Japan) equipped with a CP-Sil 8 CB column (30 m length, 0.25 mm i.d., 0.25 μ m film thickness). The injection port was set at 250 °C. The carrier gas was helium at a flow rate of 1.5 mL/min. The column oven was initially held at 40 °C for 1 min, ramped to 300 °C at 5 °C/min, and then held at 300 °C for 8 min. The mass spectrometer was operated in the scan mode over an *m/z* range of 35–300 with an emission current of 20 μ A and ionization time of 2.0 ms.

p-Dibromobenzene was used as the internal standard. Compound identification was performed by comparing the mass fragmentation patterns and retention times with authentic standards. For compounds without available standards, structures were tentatively assigned based on their MS fragmentation patterns. Yields were estimated using calibration curves derived from standard compounds based on peak area ratios relative to the internal standard.

2-(2-Hydroxyphenyl)benzofuran (9)

¹H NMR (CD₃OD, 400 MHz): δ (ppm) 6.89–6.98 (m, 2H, 3'-H, 5'-H), 7.14–7.21 (m, 2H, 6-H, 4'-H), 7.25 (dt, 1H, *J* = 1.4 Hz, *J* = 8.5 Hz, 5-H), 7.39 (d, 1H, 3-H), 7.49 (collapsed d, 1H, *J* = 7.1 Hz, 4-H), 7.58 (collapsed d, 1H, *J* = 7.5 Hz, 7-H), 7.93 (dd, 1H, *J* = 1.6 Hz, *J* = 8.2 Hz, 6'-H). ¹³C NMR (CD₃OD, 100 MHz): δ (ppm) 106.8, 111.7, 117.0, 118.9, 120.7, 122.1, 123.9, 125.1, 127.7, 130.4, 131.4, 154.3, 155.3, 156.3.

CONCLUSIONS

We report the first isolation and structural identification of 2-(2-hydroxyphenyl)benzofuran from guaiacol pyrolysis, a compound not previously found among lignin or wood pyrolyzates. Known for its potent antifungal activity, this molecule may help explain the biological effects of wood tar and pyrolygneous acid and holds potential for pharmaceutical and agricultural applications.



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