JOURNAL OF
AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2006,** *54,* 2053–2056 **2053**

Antibacterial Evaluation of 1,4-Benzoquinone Derivatives

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We investigated the antibacterial activity of some new 2-aryl-3,5-dimethoxy-1,4-benzoquinone derivatives, previously prepared from 2,6-dimethoxy-1,4-benzoquinone by Suzuki cross-coupling reactions, to find effective antibacterial agents. The antibacterial activity was first tested in vitro by the disk diffusion assay against two Gram-positive and two Gram-negative bacteria, and then the minimum inhibitory concentration (MIC) of active compounds was determined. The results showed that some 2-aryl-3,5-dimethoxy-1,4-benzoquinone derivatives inhibit growth of both Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*), microorganisms that cause food poisoning and rheumatic fever. This study points out the antibacterial activity of some 2-aryl-3,5-dimethoxy-1,4-benzoquinone derivatives.

KEYWORDS: Wood tar; quinones; 2,6-dimethoxy-1,4-benzoquinone; 2-aryl-3,5-dimethoxy-1,4-benzo-quinones; antibacterial activity

INTRODUCTION

Natural sources of novel antimicrobial agents have been sought out (*1*) especially to cure some frequently occurring bacterial food-borne illnesses (*2*) and to combat the increasing bacterial resistance to antibiotics currently used in therapeutics (*3*). In this way, a new field for antimicrobial research is emerging and there is an increased need for novel antimicrobial agents to be used as preservatives in food, beverages, cosmetics, food packing, and several industrial formulations. In Brazil, plants of *Eucalyptus* genus have been used in reforestation programs to furnish wood for paper, cellulose, and charcoal steel industries. Chromatographic analysis of *Eucalyptus* tar recovered by some stills during charcoal production shows that it is mainly constituted of phenol, 2-methoxyphenol, 2,6-dimethoxyphenol, and their alkyl derivatives (*4*), which makes *Eucalyptus* tar a valuable source for the production of 1,4-benzoquinones with good yield. 2,6-Dimethoxyphenol can be directly obtained from tar either by distillation or by separation techniques that involve methylation and oxidation of a tar fraction rich in this constituent (*4*). Quinones represent an important class of biological molecules that possess activities against several types of cancer cells (*6–8*), viruses (*9*), and fungi (*10*). Some semi-synthetic analogues of substituted 1,4-benzoquinones have in vitro cytotoxic and antioxidant activities (*11*). Medicinal agents, both naturally occurring and synthetic, have been developed on the basis of the quinone structure (*12–14*). Quinones supporting oxygenate substituents have been reported to present biological activity. For example, 2,6-dimethoxy-1,4-benzoquinone, a compound easily obtained by oxidation of wood tar fraction rich in 2,6-dimethoxyphenol (*15*) and isolated from many species of plants (*16–18*), shows in vitro significant cytotoxicity against human tumor cell lines (*18*) and exhibits antifeedant activity as well as antimicrobial and antifungal activities (*19*). 2-Methoxyphenol has been studied as the source of new derivatives with quinone-type skeleton (*5*). *Eucalyptus* tar appears to be a promising chemical source for the low cost preparation of 2,6-dimethoxy-1,4-benzoquinone (*20*). We report herein the evaluation of the antibacterial activity of 1,4-benzoquinone, 2-methoxy-, 2,6-dimethoxy-1,4-benzoquinone, and some 2-aryl-3,5-dimethoxy-1,4-benzoquinone derivatives using the halo tests against standard strains of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhimurium*, and *Escherichia coli*. The minimum inhibitory concentration (MIC) of active compounds was determined, allowing the analysis of the relationship between the molecular structure and antimicrobial activity of some of those compounds.

EXPERIMENTAL PROCEDURES

Chemicals. 1,4-Benzoquinone (**1**) and 2-methoxy-1,4-benzoquinone (**2**) were purchased from Aldrich Chemical Co. (Milwaukee, WI). 2,6-Dimethoxy-1,4-benzoquinone (**3**) was prepared from wood tar fractions (*15*). The series of 2-aryl-3,5-dimethoxy-1,4-benzoquinones (**3a–3j**) was available from our previous work and was prepared from 2,6-dimethoxy-1,4-benzoquinone by Suzuki cross-coupling reactions (*20*). Ethyl acetate (EtOAc) and dimethyl sulfoxide (DMSO) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Chloramphenicol was purchased from Sigma Chemical Co. (St. Louis, MO).

Test Strains. The test strains, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615, *Salmonella typhimurium* ATCC 13311, and *Escherichia coli* ATCC 25723, used for this study were purchased from American Type Culture Collection.

Medium. The media BHI were purchased from Merck (Darmstadt, Germany) and Biobrás (Montes Claros, Brazil). The strains were maintained on broth heart infusion (BHI) medium and refrigerated at 7 °C.

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Table 1. Antibacterial Activity of 1,4-Benzoquinones, 2-Aryl-3,5-dimethoxy-1,4-benzoquinone Derivatives, Positive Control (Chloramphenicol), and Negative Control (EtOAc) Measured by the Halo Zone Test (Units, mm)

compound[a]	corresponding effects on microorganisms			
	S. aureus	*S. pyogenes*	*S. typhimurium*	*E. coli*
1	9.1 ± 0.7	10.0 ± 0.7	11.8 ± 1.7	10.9 ± 0.7
2	15.0 ± 0.3	15.0 ± 0.2	12.0 ± 0.2	11.0 ± 0.2
3	22.8 ± 1.7	16.2 ± 1.6	13.9 ± 1.0	9.0 ± 0.3
3a	18.2 ± 1.7	12.0 ± 0.3	×[b]	×
3b	×	12.0 ± 0.6	×	×
3c	13.0 ± 0.3	×	×	×
3d	×	×	×	×
3e	16.1 ± 0.7	13.9 ± 0.7	×	×
3f	15.9 ± 1.3	12.8 ± 1.6	×	×
3g	×	×	×	×
3h	16.8 ± 1.1	10.2 ± 1.7	×	×
3i	12.0 ± 0.3	17.9 ± 1.0	×	×
3j	×	×	×	×
EtOAc	×	×	×	×
control[c]	21.0 ± 0.2	22.0 ± 0.5	30.3 ± 0.2	25.0 ± 0.2

[a] Compound (80 μg/disk) in EtOAc. [b] ×, no halo zone was formed. [c] Reference standard: chloramphenicol (30 μg/disk).

Antibacterial Assays. Antibacterial activity was detected by the disk diffusion method (*21*) with minor modifications. *S. aureus*, *S. pyogenes*, *S. typhimurium*, and *E. coli* were subcultured in BHI medium and incubated for 18 h at 37 °C, and then the bacterial cells were suspended, according to the McFarland protocol, in saline solution to produce a suspension of about 10^{-5} CFU mL^{-1}. 10 μL of this suspension was mixed with 10 mL of sterile antibiotic agar at 40 °C and poured onto an agar plate in a laminar flow cabinet. 100 μg of each test compound was dissolved in ethyl acetate (EtOAc) and added to a paper disk (6 mm diameter) that was dried and placed on the agar plate containing the bacterial cells (5 samples/disk plus control). A disk containing chloramphenicol (30 μg) and a disk containing only EtOAc were used as positive and negative controls, respectively. The susceptibility of the bacteria to the test compounds was determined by the formation of an inhibitory zone after 18 h of incubation at 36 °C. Experiments were run in triplicate, and the results are presented as mean values of the three measurements. The Student's test (at 99% level) was applied for comparing the sample means (*22*). **Table 1** reports the inhibition zones (mm) of each compound and the controls. The minimum inhibitory concentration (MIC) was evaluated by the macro dilution test using standard inoculums of 10^{-5} CFU mL^{-1}. Serial dilutions of the test compounds, previously dissolved in dimethyl sulfoxide (DMSO), were prepared to final concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, and 1 μg/mL. To each tube was added 100 μL of a 24 h old inoculum. The MIC, defined as the lowest concentration of the test compound, which inhibits the visible growth after 18 h, was determined visually after incubation for 18 h at 37 °C, and the results are presented in **Table 2**. Tests using DMSO and chloramphenicol as negative and positive controls, respectively, were carried out in parallel. All tests were performed in duplicate with full agreement between both results.

RESULTS AND DISCUSSION

The antibacterial activities of 13 1,4-benzoquinones **1**, **2**, **3**, and **3a−3j** (**Figure 1**) observed through the inhibition zone

diameters (mm) toward Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*) and Gram-negative bacteria (*Salmonella typhimurium*, *Escherichia coli*) are summarized in **Table 1**. From the 13 tested compounds, only **3d**, **3g**, and **3j** were completely inactive toward all bacteria. The MIC values for the active 1,4-benzoquinones were determined and are shown in **Table 2**. The results reveal an interesting structure-dependent activity for these compounds. From tested compounds, one is 1,4-benzoquinone **1**, two are methoxylated 1,4-benzoquinones (**2** and **3**), and 10 are 2-aryl-3,5-dimethoxy-1,4-benzoquinone derivatives. Benzoquinone **1** and methoxylated derivatives **2** and **3** were active against all four tested bacteria. The 2,6-dimethoxy-1,4-benzoquinone (**3**) was especially active against *S. aureus* with an inhibition halo of 22.8 mm of diameter, almost comparable to the size of the reference antibiotic chloramphenicol (21.0 mm). The benzoquinone **1** was active up to a dose of 32 μg mL^{-1} against *S. typhimurium* and *E. coli*, the same MIC value found for the reference drug, chloramphenicol. The introduction of one methoxy group in the 1,4-benzoquinone nucleus decreased the activity for all bacteria, except for *S. pyogenes*, which remained the same. An interesting result was observed for compound **3**, bearing two-methoxy groups. The MICs observed when 2,6-dimethoxy-1,4-benzoquinone (**3**) was tested against all strains were as good as the ones obtained with the control, except for *E. coli*, which presented a double value. The introduction of a substituted aryl group into the 2,6-dimethoxy-1,4-benzoquinone moiety led to 10 new compounds (*20*). None of those compounds was active against the Gram-negative bacteria, but compounds **3a**, **3b**, **3c**, **3e**, **3f**, **3h**, and **3i** showed selective activity against Gram-positive bacteria. Among them, compound **3b** was active only against *S. pyogenes*, while compound **3c** showed action only against *S. aureus*. This is an interesting effect of the halogen identity (bromo in **3b** and fluor in **3c**) as the *para*-substituents of aromatic rings. In a number of cases, it has been postulated that the antibacterial activity can be increased when a highly electronegative arrangement is present in the molecule, such as an extended conjugated system (*23*) like the ones present in compounds **3d**, **3g**, and **3j**. Surprisingly, when the electron withdrawal groups such as NO$_2$ (**3d**), CHO (**3g**), and CN (**3j**) were introduced in the para position, compounds became completely inactive toward all tested bacteria, inferring the necessity of an atom rich in electrons in such position to bind to an active site of the bacteria and then to promote activity. The absence of activity of **3d**, **3g**, and **3j** could also depend on the size of the substituent in the para position. However, Yatagai et al. (*24*) reported that there is only a very discrete difference in the termiticidal activity of some relative phenolic derivatives upon variation of the para substituent size. This behavior can be explained by taking into account the whole molecule. 1,4-Benzoquinones are a special example of cross-conjugated enediones. Their ability to accept one electron initially, forming the semiquinone anion radical, followed by a further electron to give the dianion, is the dominant feature of quinone chemistry. It is this reversible

Table 2. Minimal Inhibitory Concentration (MIC) of the Selected Compounds

MIC (μg mL^{-1}) strain	compound										
	1	**2**	**3**	**3a**	**3b**	**3c**	**3e**	**3f**	**3h**	**3i**	C[a]
S. aureus	64	128	32	128	−[b]	128	128	256	128	64	32
S. pyogenes	64	64	32	64	64	−	128	64	64	32	32
S. typhimurium	32	64	32	−	−	−	−	−	−	−	32
E. coli	32	128	64	−	−	−	−	−	−	−	32

[a] Chloramphenicol. [b] −not tested.

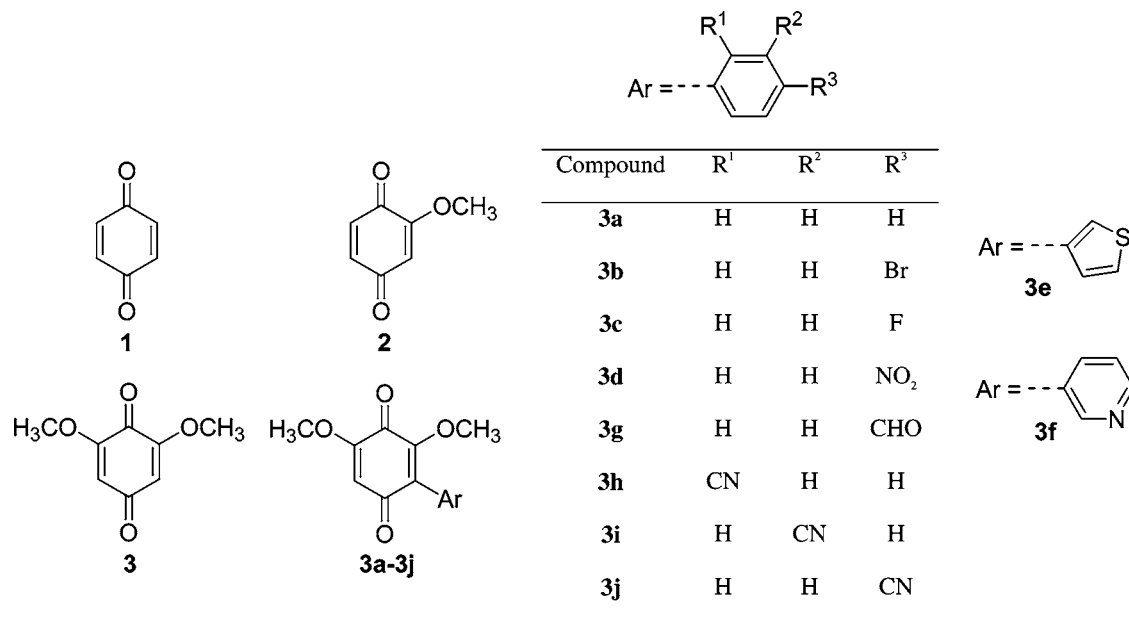| Compound	R^1	R^2	R^3
3a	H	H	H
3b	H	H	Br
3c	H	H	F
3d	H	H	NO₂
3g	H	H	CHO
3h	CN	H	H
3i	H	CN	H
3j	H	H	CN

Figure 1. Chemical structures of 2-aryl-3,5-dimethoxy-1,4-benzoquinones and related compounds.

reduction process that accounts for the biological activity (*25, 26*). In our case, the extended conjugation promoted by the substituents CN, CHO, and NO₂ in the para position led the molecule to have a partial positive charge on the carbonyl group of the quinone moiety, deactivating the major electronegative center of the molecule (quinone moiety). An additional valuable structure−activity relationship was noticed by comparing results obtained for compounds **3h**, **3i**, and **3j**, all bearing a CN group in the aromatic ring. From the three isomers, only two of them (ortho and meta substituted derivatives **3h** and **3i**, respectively) were active. This fact shows that the effect of a CN group is only responsible for the loss of activity when it occupies the para position. This again points out the importance of the identity of the substituent in this para position for an effective antibacterial activity. The results also suggest that the introduction of a bulk group in the molecule alters the coplanarity of the methoxy group toward the quinone ring, and this change may be responsible for the resulting selectivity.

Even though the results show that most of the tested 2-aryl-3,5-dimethoxy-1,4-benzoquinones have lower antibacterial activities than observed for the reference drug, chloramphenicol, results are promising because the disk diffusion test is carried out in an aqueous environment and the test compounds have poor diffusion rates on agar and are insoluble in water (*27*).

The present study revealed a new class of 2-aryl-3,5-dimethoxy-1,4-benzoquinone derivatives with selective antibacterial activity against microorganisms that cause food poisoning (*28*) and rheumatic fever (*26*) and points out again the importance of the quinonoid system for biological activity of these compounds. The inhibitory mechanism of quinone compounds in Gram-positive bacteria seems to be an attractive subject for further investigations.

It is interesting to observe that the relative ease of preparation of active benzoquinone derivatives from wood tar, reported herein, makes such compounds of distinguished interest for both food and pharmaceutical industries where economically interesting compounds could be synthesized from waste materials. The toxicity of benzoquinone derivatives and structurally similar compounds is not well defined, but seems to be low enough to permit the development of new antimicrobials for human use or as agents to prevent bacterial food spoilage.

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