DNA Cleavage by Di- and Trihydroxyalkylbenzenes. Characterization of Products and the Roles of O_2, Cu(II), and Alkali

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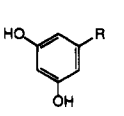
Abstract: Several 5-alkyl-1,3-dihydroxybenzene (5-alkylresorcinol, **1**) and 6-alkyl-1,2,4-trihydroxybenzene (**2**) derivatives were prepared and used to study the mechanism by which such compounds effect Cu(II)-dependent DNA strand scission. Comparison of the methyl, *n*-pentyl, *n*-undecyl, and *n*-hexadecyl derivatives in each structural series indicated that the efficiency of DNA cleavage increased with increasing length of the alkyl substituent. DNA cleavage by the 5-alkylresorcinols appears to involve initial oxygenation of the benzene nucleus, a process that occurs readily at alkaline pH in the presence of Cu^{2+} and O_2. The resulting trihydroxylated benzenes mediate DNA cleavage in a reaction dependent on the presence of both Cu^{2+} and O_2. The mechanism appears to involve reduction of Cu^{2+} by the trihydroxybenzene moiety in **2**, with subsequent formation of reactive oxygen species. The ability of catalase and dimethyl sulfoxide to suppress DNA strand scission is consistent with the intermediacy of H_2O_2 and $^{\bullet}OH$ in the DNA strand scission process.

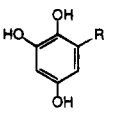
5-Alk(en)yl-1,3-dihydroxybenzenes, commonly known as resorcinols, have been isolated from many plants, including those of the Proteaceae, Anacardiaceae, Ginkogoaceae, and Graminae families.[1] The resorcinols display a wide variety of biological activities, including fungicidal and bactericidal activities against numerous pathogens.[2] Certain resorcinols have been reported to inhibit the growth of cultured human erythrocytes and chick embryo cells derived from heart tissue.[3] Their presence causes lesions in T-lymphocyte chromosomes[4] and results in the clustering of cells in culture.[5] Resorcinols have also been shown to inhibit a number of enzymes including trypsin,[6] tryptophan peroxidase,[7] and tyrosinase.[8] Topical application of resorcinols to derma causes blistering[9] but can also retard, although not arrest, polycyclic aromatic hydrocarbon-induced tumorigenesis.[10]

Recently, we reported that several naturally occurring 5-alk(en)ylresorcinols were able to effect Cu(II)-dependent relaxation of supercoiled plasmid DNA.[11] Although the mechanisms of DNA binding and cleavage by these compounds were uncertain, the novelty of the observations and simplicity of the structures involved has prompted a more detailed investigation of the nature of these processes.

Presently, we describe the preparation and mechanistic evaluation of 5-alkyl-1,3-dihydroxybenzene (5-alkylresorcinols, **1**) and 6-alkyl-1,2,4-trihydroxybenzene (**2**) derivatives, including



1a R = CH_3
1b R = *n*-C_5H_{11}
1c R = *n*-$C_{11}H_{23}$
1d R = *n*-$C_{16}H_{33}$



2a R = CH_3
2b R = *n*-C_5H_{11}
2c R = *n*-$C_{11}H_{23}$
2d R = *n*-$C_{16}H_{33}$

those containing methyl, *n*-pentyl, *n*-undecyl, and *n*-hexadecyl substituents. We demonstrate that DNA binding is mediated by the alkyl substituents and becomes more efficient as the length of the chain increases. Evidence is provided which suggests that DNA cleavage occurs following initial oxygenation of the benzene nucleus in the resorcinols, a process that is shown to be facilitated at alkaline pH by the presence of Cu^{2+} and O_2. The resulting trihydroxylated alkylbenzene derivatives mediate DNA cleavage that is dependent on the presence of Cu^{2+} and O_2; the mechanism seems to involve reduction of Cu^{2+} by the benzene moiety, with the subsequent generation of diffusible oxygen radicals. DNA cleavage per se is effected by the reactive oxygen species so generated, resulting in the release of free bases and the production of DNA fragments having 5′-phosphates and 3′-phosphates or 3′-phosphoroglycolates.

Results

Syntheses of 5-Alkyl-1,3-dihydroxybenzenes and 6-Alkyl-1,2,4-trihydroxybenzenes. The syntheses of 5-alkyl-1,3-dihydroxybenzenes **1c,d** were accomplished by a straightforward route (supporting information, Scheme 1). This involved

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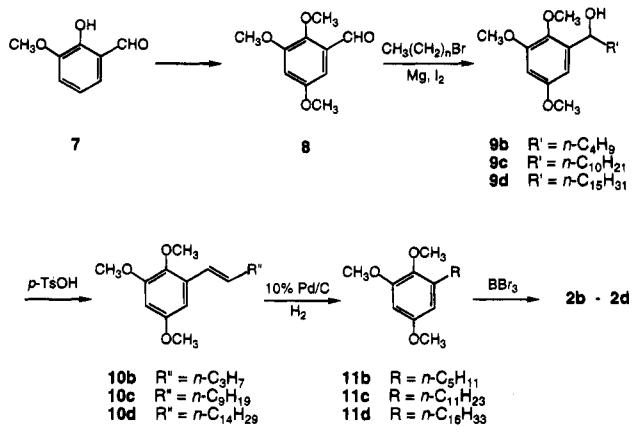
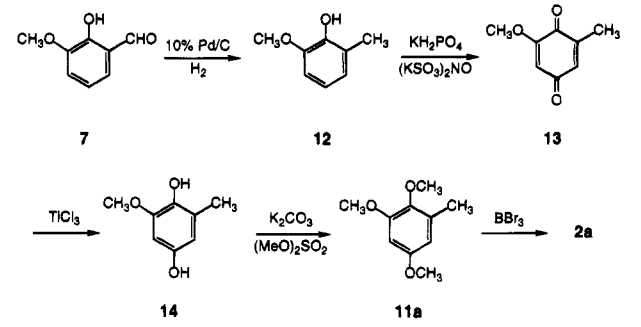
Scheme 1



Scheme 2



condensation of 3,5-dimethoxybenzaldehyde (**3**) with the appropriate alkylmagnesium reagents to afford benzyl alcohol derivatives **4c,d**. Following dehydration of the alcohols via the agency of *p*-toluenesulfonic acid, the 3,5-dimethoxystyrene derivatives so obtained (**5c,d**) were converted to the respective 5-alkyl-1,3-dimethoxybenzenes (**6c,d**) in good yield by catalytic hydrogenation over 10% palladium-on-carbon. Deblocking of the OCH_3 groups (BBr_3, CH_2Cl_2) then afforded **1c,d**, which were isolated as colorless solids. The overall yields of the final products were 28% for **1c** and 40% for **1d**.

The preparation of 6-alkyl-1,2,4-trihydroxybenzenes **2b−d** was carried out analogously, starting from 2,3,5-trimethoxybenzaldehyde (Scheme 1). The latter was accessible by Elbs persulfate oxidation[12] of *o*-vanillin (**7**), followed by methylation of the derived hydroxylated intermediate. Although **8** could be obtained only in low overall yield by this procedure, the large scale on which this transformation could be run, and the propensity of **8** to crystallize after chromatographic purification, permitted **8** to be obtained with reasonable facility in quantities sufficient for the purposes of this study. The conversions **8** → **9** → **10** → **11** → **2** were carried out in the same fashion described above for the preparation of **1c,d**. Intermediates **11b−d** were demethylated by the use of BBr_3 (CH_2Cl_2, 0 → 25 °C), providing the desired 6-alkyl-1,2,4-trihydroxybenzenes (**2b−d**). The overall yields of these three compounds from benzaldehyde derivative **8** were 20−73%. The trihydroxybenzenes were unstable and tended to oxidize rapidly, especially in solution.

The synthesis of 2,3,5-trihydroxytoluene (**2a**) was also effected by starting from *o*-vanillin (**7**) (Scheme 2). Hydrogenation of **7** over 10% palladium-on-carbon provided 2-hydroxy-3-methoxytoluene (**12**) in 98% yield, a transformation that presumably proceeds in two stages via the intermediacy of an initially formed benzyl alchohol derivative. As shown in Scheme 2, toluidine derivative **12** was then converted to 2-methoxy-6-methyl-1,4-benzoquinone (**13**) in 70% yield by treatment with potassium nitrosodisulfonate (Fremy's salt).[13] The quinone, which was isolated as fine yellow needles, was converted to the respective hydroquinone (**14**) in essentially quantitative yield via the agency of $TiCl_3$.[14] The hydroquinone was methylated (K_2CO_3, dimethyl sulfate, acetone) to provide 2,3,5-trimethoxytoluene (**11a**). Compound **11a** was isolated as a low melting, crystalline product following chomatographic purification. Demethylation under the usual conditions (BBr_3, CH_2Cl_2) then afforded 2,3,5-trihydroxytoluene (**2a**) as colorless needles. All compounds described above were characterized spectroscopically and by combustion analysis or high-resolution mass spectrometry.

DNA Relaxation Assays. The ability of individual di- and trihydroxyalkylbenzenes to effect DNA cleavage was assayed by the use of ϕX174 replicative form DNA, a supercoiled, covalently closed circular DNA (Form I DNA). The creation of a break on either DNA strand effects relaxation of the supercoils and conversion to a nicked (Form II) DNA readily separable from Form I by electrophoresis on agarose gels. DNA breaks on both strands within close proximity produce linear duplex (Form III) DNA, which is readily separable from Forms I and II. Because a single nick at any site within the 5386 base pairs of ϕX174 replicative form DNA effects its conversion to Form II, the assay is highly sensitive.[11]

The use of this assay for the measurement of DNA nicks produced by 1,3-dihydroxy-5-methylbenzene (orcinol, **1a**) in the presence of Cu^{2+} is illustrated in Figure 1 of the supporting information. Compound **1a** (100 μM) + 30 μM Cu^{2+} produced significant conversion of Form I → Form II DNA, relative to a reaction in which **1a** was omitted (cf. lanes 3 and 7). It was also possible to demonstrate that the extent of DNA relaxation increased with increasing concentrations of **1a** employed (cf. lanes 7, 11, and 15), as well as the concentration of Cu^{2+} (e.g., lanes 7−10). Also included in the assay were controls that employed DNA alone (lane 1), or DNA + Fenton's reagent, which is a source of reactive oxygen species (lane 2).

Definition of Parameters Conducive to DNA Cleavage. Because it has been noted previously[11] that the ability of individual 5-alkylresorcinol derivatives to cleave DNA increased substantially when they were maintained in alkaline solution, we sought to establish the nature of the chemical changes that might be expected to obtain under such conditions. We also found that the increase in the DNA cleaving ability of the compounds was enhanced if they were incubated in the presence of Cu^{2+} (*vide infra*), providing a further clue as to the nature of the chemical transformation(s). Havinga and his co-workers demonstrated that a number of phenolic compounds underwent hydroxylation *ortho* to the existing phenolic OH group when treated with Cu^{2+} + an amine.[15] The reported conversions were surprisingly efficient and prompted us to consider whether the DNA cleaving potential of our 5-alkylresorcinols (5-alkyl-1,3-dihydroxybenzenes) might also result from initial oxygenation of the aromatic nucleus. Accordingly, 1,3-dihydroxy-5-methylbenzene (**1a**) was incubated in aqueous solution in the presence of Cu^{2+} and *n*-amylamine and the solution was aerated for 5 min. As shown in Figure 2 of the supporting information,

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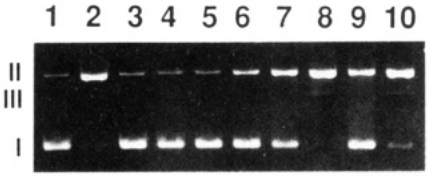

Figure 1. Effect of base treatment on DNA cleavage by 1,3-dihydroxy-5-methylbenzene (**1a**) and 6-methyl-1,2,4-trihydroxybenzene (**2a**): lane 1, DNA alone; lane 2, 0.03% H_2O_2 + 10 μM Fe^{2+}; lane 3, 20 μM **1a**; lane 4, 20 μM **2a**; lane 5, 20 μM base-treated **1a**; lane 6, 20 μM base-treated **2a**; lane 7, 20 μM **1a** + 20 μM Cu^{2+}; lane 8, 20 μM base-treated **1a** + 20 μM Cu^{2+}; lane 9, 20 μM base-treated **2a** + 20 μM Cu^{2+}; lane 10, 20 μM **2a** + 20 μM Cu^{2+}.

the sample of **1a** so treated was substantially more efficient at mediating DNA relaxation (54% Form II DNA in the presence of 50 μM **1a**) than a sample of **1a** that had been incubated with Cu^{2+} alone (30% Form II) or *n*-amylamine alone (30% Form II), or which had not received any treatment prior to assay 28% (Form II). Although most of the experiments carried out in this study for resorcinol derivatives **1** utilized alkaline conditions in the absence of an amine, there is ample precedent for the aerobic oxidation of such species under comparable conditions.[16]

Because this experiment was consistent with the possible intermediacy of a (tri)hydroxylated species that was actually responsible for DNA cleavage by the 5-alkylresorcinols, we carried out a direct comparison of a few sets of di- and trihydroxylated alkylbenzenes having the same alkyl substituents. As shown in Figure 3 of the supporting information, when ϕX174 replicative form DNA was incubated in the presence of 25 μM 6-undecyl-1,2,4-trihydroxylbenzene (**2c**) + 100 μM Cu^{2+}, the efficiency of DNA relaxation (82% Forms II and III DNA) was much greater than that observed for the corresponding 5-undecylresorcinol (**1c**) (38% Form II DNA). The analogous result was obtained for direct comparisons of **1a** vs **2a**, **1b** vs **2b**, and **1d** vs **2d** (data not shown), supporting the thesis that Cu-dependent DNA cleavage by these 5-alkylresorcinols may involve initial hydroxylation of the benzene ring.

In the foregoing comparisons, it was noted that the trihydroxybenzene derivatives were always more efficient than the respective dihydroxybenzenes in mediating DNA cleavage. Interestingly, it was also found that within a given structural series (i.e., all dihydroxybenzenes (**1a**−**d**) or all trihydroxybenzenes (**2a**−**d**)) DNA cleavage activity varied substantially and seemed to increase with increasing alkyl chain length. This is illustrated in Figure 4 of the supporting information, which depicts the relaxation of Form I DNA in the presence of 30 μM Cu^{2+} + 10 μM 6-pentyl-1,2,4-trihydroxybenzene (**2b**) or 10 μM 6-hexadecyl-1,2,4-trihydroxybenzene (**2d**). Densitometric analysis of the agarose gel indicated that **2d** had effected conversion of the substrate DNA → Form II in 81% yield, while the comparable figure for **2b** was 28%. Analogous differences were noted for several other direct comparisons within structural series **2** and also for 5-alkyl-1,3-dihydroxybenzene derivatives **1** (data not shown).

In order to characterize the chemical events leading to DNA strand scission, we studied the effects of alkali, O_2, and Cu^{2+} in greater detail. As shown in Figure 1, pretreatment of 1,3-dihydroxy-5-methylbenzene (**1a**) with alkali under aerobic conditions greatly potentiated its ability to cleave DNA (cf. lanes 7 and 8), consistent with the suggestion that this species must be oxygenated on the benzene nucleus prior to effecting DNA cleavage. Since this putative hydroxylation is believed to effect its conversion to 6-methyl-1,2,4-trihydroxybenzene (**2a**), analogous pretreatment of authentic **2a** with alkali should logically

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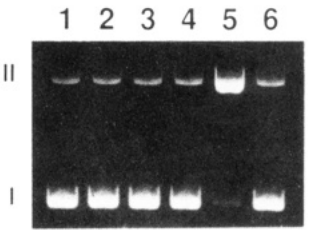

Figure 2. DNA cleavage by 1,3-dihydroxy-5-methylbenzene (**1a**) + Cu^{2+} base treated in the presence and absence of O_2. All reactants were added to the DNA cleavage reactions simultaneously, which were then monitored under ambient conditions for 30 min prior to analysis by agarose gel electrophoresis: lane 1, DNA alone; lane 2, 200 μM base-treated sample of Cu^{2+}; lane 3, 100 μM base-treated **1a**; lane 4, 100 μM **1a** + 200 μM Cu^{2+}; lane 5, 100 μM **1a** + 200 μM Cu^{2+}, which were base treated together aerobically; lane 6, 100 μM **1a** + 200 μM Cu^{2+}, which were base treated together anaerobically.

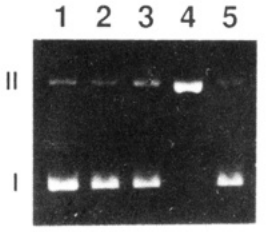

Figure 3. Effect of dioxygen on DNA cleavage by 6-methyl-1,2,4-trihydroxybenzene (**2a**). All reactants were incubated for 20 min in the presence (lanes 1−4) or absence (lane 5) of O_2; lane 1, DNA alone; lane 2, 100 μM Cu^{2+}; lane 3, 20 μM **2a**; lane 4, 20 μM **2a** + 100 μM Cu^{2+}; lane 5, 20 μM **2a** + 100 μM Cu^{2+}.

not improve its ability to cleave DNA. In fact, actual treatment of **2a** with base was found to slightly diminish its ability to mediate Cu^{2+}-dependent DNA cleavage (cf. lanes 9 and 10), consistent with the suggestion that alkali was not required for any mechanistic process subsequent to hydroxylation of the benzene ring. Also consistent with this view was the finding (supporting information, Figure 5) that alkali pretreatment enhanced the potency of 1,3-dihydroxy-5-methylbenzene (**1a**) as a DNA cleaving agent only when Cu^{2+} was also present during the alkali treatment (74% vs 40% Form II DNA, cf. lanes 5 and 6). Presumably, Cu^{2+} + alkali functions in much the same fashion as Cu^{2+} + *n*-amylamine (supporting information, Figure 2) in facilitating hydroxylation of the benzene ring.[15,16] The role of O_2 in this putative aromatic hydroxylation reaction was also studied. As shown in Figure 2, pretreatment of **1a** with alkali + Cu^{2+} was effective in potentiating the DNA cleaving activity of this compound only when this treatment was also carried out in the presence of O_2 (cf. lanes 5 and 6). One obvious possibility is that the oxygen atom putatively incorporated into the aromatic nucleus is actually derived from dioxygen.[15,16]

Shown in Figure 3 are the results of an experiment designed to determine whether O_2 was also required for DNA cleavage by 5-alkylresorcinols subsequent to the putative hydroxylation of the aromatic nucleus. As is clear from the figure, DNA cleavage by 6-methyl-1,2,4-trihydroxybenzene (**2a**, which presumably arises *in situ* as **1a** is hydroxylated) was absolutely dependent on the presence of both Cu^{2+} and O_2. Thus, unlike alkali, which was associated only with the oxidative transformation of the benzene nucleus in **1**, Cu^{2+} and O_2 were required both for "activation" of the DNA cleaving agent (i.e., **1** → **2**) and as participants in subsequent chemical events essential for DNA cleavage per se.

The nature of these latter events was also investigated. Neocuproine is a dimethylated phenanthroline derivative that

binds Cu(I) much more efficiently than Cu(II).[17] As shown in Figure 6 of the supporting information, neocuproine completely inhibited DNA cleavage mediated by 6-methyl-1,2,4-trihydroxybenzene (**2a**) and also by 1,3-dihydroxy-5-methylbenzene (**1a**), suggesting that Cu(I) is an obligatory intermediate on the pathway to DNA strand scission. The source of electrons for the reduction of Cu(II) → Cu(I) would presumably be the (oxygenated) benzene nucleus; thus Cu(I) would be formed reductively as the trihydroxylated aromatic nucleus was oxidized to quinonoid products.[15,16] Consistent with this suggestion was the observation that the oxidant DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone), which is known to be capable of oxidizing hydroquinones to quinones,[18] eliminated the ability of 6-methyl-1,2,4-trihydroxybenzene (**2a**) to effect Cu-dependent DNA cleavage when DDQ and **2a** were incubated together for 30 min prior to the DNA cleavage assay (supporting information, Figure 7). An analogous observation was made when **2a** was assayed for DNA cleavage after preincubation with tyrosinase, an enzyme that effects the oxidation of catechol moieties to *o*-quinones.[15,19] As shown in Figure 8 of the supporting information, tyrosinase eliminated the ability of **2a** to effect subsequent DNA cleavage in the presence of Cu^{2+}.

The finding that Cu(I) is an obligatory intermediate in DNA cleavage mediated by **2a** and that O_2 is also required suggested that the reactive species actually responsible for DNA strand scission may be one or more oxygen radicals generated from O_2 at the metal center. In fact, the DNA cleavage mediated by **2a** + Cu^{2+} was completely suppressed when the incubation mixture also contained dimethyl sulfoxide, a known scavenger of $^{\bullet}OH$[20] (supporting information, Figure 9). Also of interest in this context was the observation made when a DNA cleavage assay involving **2a** + Cu^{2+} was carried out in the presence of catalase, an enzyme that disproportionates H_2O_2 to afford H_2O + O_2.[21] As shown in Figure 8 of the supporting information, catalase suppressed DNA cleavage by **2a** + Cu^{2+}.

Analysis of Products of DNA Cleavage. The nature of the DNA cleavage products resulting from treatment with representative di- and trihydroxyalkylbenzenes was studied initially using a number of DNA restriction fragments. Shown in Figure 4 is the cleavage by 6-(*n*-pentyl)-1,2,4-trihydroxybenzene (**2b**) of a 5′-^{32}P end labeled DNA restriction fragment 149 base pairs in length. As is clear from the figure, DNA degradation occurred at every position within the DNA duplex. Moreover, the extent of cleavage increased both with increasing time and with increasing concentrations of **2a**. Similar results were obtained with a number of the di- and trihydroxyalkylbenzenes; the efficiency of cleavage of the end-labeled restriction fragments correlated reasonably with the potencies of individual compounds in mediating the relaxation of supercoiled DNA's (*vide supra*).

The mobilities of the bands in Figure 4 were the same as those of the Maxam−Gilbert sequencing bands, the latter of which have phosphate groups at their 3′-termini. In other experiments that employed 20% polyacrylamide gels, the cleavage of 5′-^{32}P end labeled DNA's by the di- and trihydroxyalkylbenzenes was shown to produce two bands at each nucleotide position. These co-migrated with bands produced from the same substrates by Fe(II)·BLM (Fe(II)·bleomycin) and Fe(II)·MPE (methidiumpropyl-EDTA-iron(II)). Treatment of 3′-^{32}P end labeled DNA duplexes with the di- and trihydroxyalkylbenzenes afforded bands on polyacrylamide gels that co-migrated with products of Maxam−Gilbert sequencing reactions, indicating the presence of 5′-phosphate groups.

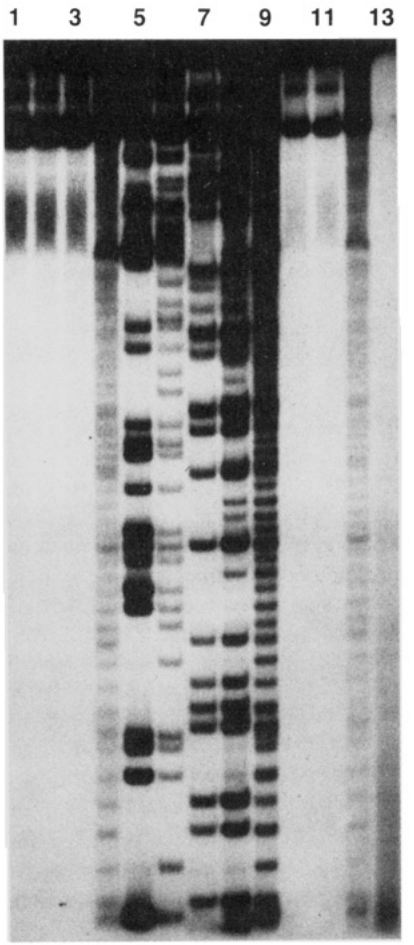

Figure 4. Cleavage of a linear DNA duplex by 6-(*n*-pentyl)-1,2,4-trihydroxybenzene (**2b**) in the presence of Cu^{2+}. A 5′-^{32}P end labeled 149-bp DNA restriction fragment was incubated with 20 μM Cu^{2+} and varying concentrations of **2b** at 25 °C for 1 h (lanes 1−4) or 2 h (lanes 10−13), then analyzed on a 10% denaturing polyacrylamide gel: lanes 1 and 10, 50 μM **2b** + Cu^{2+}; lanes 2 and 11, 100 μM **2b** + Cu^{2+}; lanes 3 and 12, 200 μM **2b** + Cu^{2+}; lanes 4 and 13, 2 mM **2b** + Cu^{2+}; lane 5, Maxam−Gilbert G lane; lane 6, G + A lane; lane 7, C lane; lane 8, C + T lane; lane 9, Fe(II)·MPE.

Table 1. Quantification of Products Resulting from Treatment of 5′-d($CGCT_3A_3GCG$)-3′ with Cu^{2+} + **2a**

base	amount (μM)
cytosine	4.9
thymine	4.4
adenine	5.3
guanine	5.1

A more detailed analysis of the chemistry of DNA degradation was carried out using the self-complementary dodecanucleotide 5′-d($CGCT_3A_3GCG$)-3′ as a substrate. As shown in Table 1, the monomeric products were limited to the four nucleic acid bases, which were produced in roughly equal amounts. The equal distribution of bases released, and the absence of any predominant oligonucleotide produced by site-selective cleavage, reinforced the observations made in Figure 4 regarding lack of sequence selectivity.

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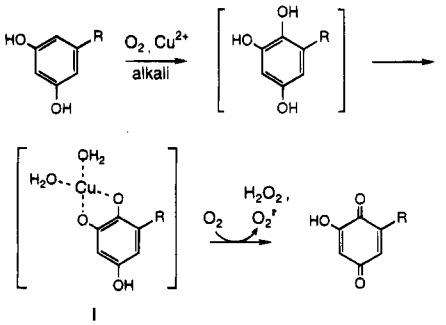
Discussion

We have previously reported the isolation of several 5-alk(en)ylresorcinols from extracts prepared from the plants *Hakea trifurcata* and *Hakea amplexicaulis*.[11] These compounds were isolated by bioassay-guided fractionation on the basis of their ability to nick DNA in the presence of Cu^{2+}. As noted previously,[11] fractions of the crude extracts were characterized by the unusual observation that, after individual steps in the fractionation scheme, none of the fractions had significant DNA nicking activity initially, but the activity "reappeared" as the fractions were maintained in solution under ambient conditions. Also of interest in this regard were the isolated agents themselves, which mediated Cu(II)-dependent DNA cleavage at 10 μM concentration in spite of the absence of good ligands for Cu(II)! Both observations suggested that the 5-alk(en)-ylresorcinols were probably transformed chemically to some other species, the latter of which was actually responsible for mediating DNA strand scission.

Initial experiments with the 5-alkylresorcinols indicated that the efficiency of DNA cleavage was enhanced substantially if the agents were maintained in aqueous solution under somewhat alkaline conditions prior to assaying for DNA strand scission. The effect of alkali was particularly pronounced when Cu^{2+} was also present. This finding was reminiscent of the reports by Havinga and his co-workers,[15] who found that oxygenation of a number of phenolic compounds was facile in the presence of Cu^{2+} and an amine. In fact, when a solution of orcinol (**1a**) containing Cu^{2+} + *n*-amylamine was aerated for 5 min, its ability to effect DNA strand scission was enhanced considerably (supporting information, Figure 2).

While the literature contains ample precedent for the oxidation of phenolic compounds under basic conditions and in the presence of strong oxidants,[16] fewer studies have involved conditions analogous to those employed in the DNA cleavage assays that resulted in isolation of the 5-alk(en)ylresorcinols as DNA cleaving agents. However, *p*-methoxyphenols and 6-chromanols underwent *ortho*-acetoxylation when treated with $Cu(OAc)_2$ in acetic acid,[22] phenols have been converted to the respective *para*-quinones with $CuCl_2$ and O_2 in the presence of hydroxylamine[23] or alcohols,[24] and benzene underwent conversion to hydroquinone in the presence of CuCl + O_2.[25] Therefore, it seemed reasonable to carry out experiments to assess the possible intermediacy of a hydroxylated 5-alkylresorcinol as the species responsible for Cu(II)-dependent DNA strand scission. Accordingly, we compared 5-undecylresorcinol (**1c**) and 6-undecyl-1,2,4-trihydroxybenzene (**2c**)[26] as DNA cleaving agents in the presence of Cu(II). Compound **2c** was much more efficient than the corresponding resorcinol derivative **1c**; the same pattern was evident when 6-alkyl-1,2,4-trihydroxybenzenes **2a**, **2b**, and **2d** were compared with the respective 5-alkylresorcinols (**1a**, **1b**, and **1d**) (Figure 3, supporting information). Over a range of experimental conditions, the 6-alkyl-1,2,4-trihydroxybenzenes tested were 50−100 times more potent in effecting relaxation of supercoiled DNA than

Scheme 3



the respective 5-alkyl-1,3-dihydroxybenzenes (5-alkylresorcinols, **1**). The greater efficiency of compounds **2** in mediating DNA strand scission is altogether consistent with Scheme 3, which posits the conversion of the 5-alkylresorcinols to the respective 6-alkyl-1,2,4-trihydroxybenzenes.

Scheme 3 envisions the hydroxylation of the 5-alkylresorcinol substrate as a process that requires Cu^{2+} and O_2. These two points were established experimentally by showing that incubation of 1,3-dihydroxy-5-methylbenzene (**1a**) under alkaline conditions facilitated subsequent DNA cleavage only if both Cu^{2+} (Figure 5, supporting information) and O_2 (Figure 2) were present during the incubation. It may be noted that the intermediacy of **i** in the scheme also accommodates the known requirement for Cu^{2+} in 5-alkyresorcinol-mediated DNA strand scission,[11] as it provides a species that can act as a bidentate ligand for Cu(II).

Unlike alkali, whose role is depicted appropriately in Scheme 3 as being limited to the putative hydroxylation reaction (cf. Figure 1), additional roles in DNA strand scission are envisioned for Cu^{2+} and O_2. According to the Scheme, Cu^{2+} would undergo reduction to Cu^+ with concomitant oxidation of the catechol moiety in **i**, a transformation for which there is ample chemical precedent.[27] Consistent with this scheme was the finding that neocuproine, a Cu^+-selective binding agent, completely suppressed DNA cleavage by 6-methyl-1,2,4-trihydroxybenzene, as well as by 1,3-dihydroxy-5-methylbenzene (Figure 6, supporting information). This finding, which suggests strongly that Cu^+ is a required intermediate in the process that results in DNA strand scission, has been reinforced by the explicit isolation and characterization of a crystalline Cu^+−neocuproine complex.[28] Additional support derives from the finding that pretreatment of 6-methyl-1,2,4-trihydroxybenzene (**2a**) with either 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) or tyrosinase eliminated the ability of **2a** to cleave DNA in the presence of Cu^{2+} (Figures 7 and 8, supporting information), presumably by oxidation of the intermediate catechol moiety. Further, it may be noted that urushiol components, which are 3-alk(en)ylcatechol derivatives, also mediated DNA cleavage in the presence of $CuCl_2$ + O_2[29] but that an *O*-methylated alklyresorcinol derivative that presumably could not form a catechol derivative by a process analogous to that described here did not cleave DNA in the presence of Cu^{2+} + O_2.[30]

The role of oxygen in at least one step subsequent to the putative hydroxylation reaction outlined in Scheme 3 was

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(26) Although hydroxylation could, in principle, also afford the respective 5-alkyl-1,2,3-trihydroxybenzenes, isolation of the products resulting from treatment of orcinol with Cu^{2+} + O_2 under alkaline conditions suggests that intermediates involving oxygenation adjacent to the alkyl group predominate (Singh, U. S.; Hecht, S. M. Unpublished data).
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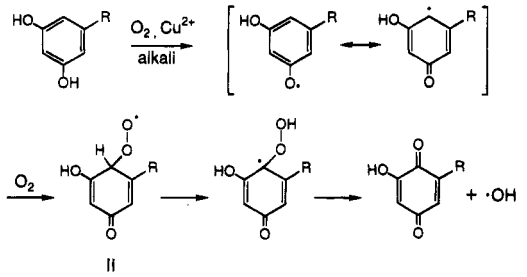
Scheme 4



II

supported by the finding that 6-methyl-1,2,4-trihydroxybenzene (**2a**) would not effect DNA cleavage in the presence of Cu^{2+} unless O_2 was present (Figure 3). One obvious role for O_2 might be in the generation of diffusible oxygen radicals such as •OH, which could be the actual mediators of DNA destruction. It has recently been demonstrated that •OH is formed from H_2O_2 in the presence of Cu^{2+};[31] the observation that catalase (Figure 8, supporting information) and DMSO (Figure 9, supporting information) both suppressed Cu^{2+}-mediated DNA strand scission in the present case suggests that H_2O_2 and •OH are both participants in the overall strand scission process.[32]

As described above, the analysis of DNA products formed by di- and trihydroxyalkylbenzenes in the presence of Cu^{2+} and O_2 has been carried out using ^{32}P end labeled DNA restriction fragments. Degradation of 5′-^{32}P end labeled DNA's afforded products that co-migrated with products of Fe(II)•BLM- and Fe(II)•MPE-mediated degradation, the latter of which are known[33,34] to terminate with 3′-phosphates and 3′-phosphoroglycolates. Although treatment of DNA with higher concentrations of compounds **1** and **2** did not afford products nearly comparable in amount to those produced by agents such as Fe(II)•BLM and Fe(II)•MPE, HPLC analysis did reveal the production of all four nucleic acid bases in roughly equal amounts. Thus, the DNA products formed by the di- and trihydroxyalkylbenzenes are the same as those produced by agents that mediate oxidative DNA damage.

Unlike Fe(II)•BLM, which produces both free bases and base propenals concomitant with DNA degradation,[33] the di- and trihydroxyalkylbenzenes studied here produced only free bases. This is the same as the products produced by Fe(II)•MPE,[34] an EDTA derivative that seems likely to mediate DNA degradation via the agency of •OH.[35] Thus the DNA degradation products observed are fully consistent with the production of •OH as the reactive species that mediates DNA degradation by **1** and **2** in the presence of Cu^{2+} (cf. Scheme 3).

It may be noted that, in addition to DNA cleavage, the quinone "byproduct" whose formation is envisioned by Schemes 3 and 4 might well be expected to alkylate DNA.[36,37] In fact, attachment of a 1-alkyl-3,4-dihydroxybenzene derivative to an antisense oligonucleotide resulted in alkylation of the target oligonucleotide upon admixture of Cu^{2+}.[38]

Although Scheme 3 is consistent with all available experimental evidence for the cleavage of DNA by 5-alkylresorcinols and 6-alkyl-1,2,4-trihydroxybenzenes, it may be noted that at least one other mechanism accommodates much of the available data as well. Scheme 4 outlines a one-electron oxidation of the 5-alkylresorcinol, followed by reaction with O_2 to form a hydroperoxy radical (**ii**). Rearrangement of this intermediate, and homolytic cleavage of the peroxide bond, affords •OH and the same hydroxyquinone product whose formation is envisioned according to Scheme 3. This scheme thus provides the same final product derived from 5-alkyresorcinol as does Scheme 3, as well as •OH for mediating DNA strand scission, but does not invoke the intermediacy of a 6-alkyl-1,2,4-trihydroxybenzene intermediate. While consistent with much of the available experimental data, the finding that neocuproine suppresses DNA strand scission mediated by orcinol (**1a**) + Cu^{2+} seems inconsistent with Scheme 4, since Cu^{+} is not required for the generation of •OH according to this scheme. Nonetheless, it may be worth noting that •OH, generated by admixture of Cu^{+} + H_2O_2, has been shown to effect the hydroxylation of benzene,[39] so that the chemical transformations which obtain upon admixture of Cu^{2+} and a 5-alkylresorcinol are likely to include processes not represented in Scheme 3.

Also worthy of note is the source of DNA affinity in the 5-alkylresorcinols (**1**) and 6-alkyl-1,2,4-trihydroxybenzenes. As illustrated in Figure 4 of the supporting information for compounds **2b,d**, the efficiency of DNA cleavage was proportional to the length of the alkyl substituent, an observation that proved to be general for all tested compounds of types **1** and **2**. Although the association of lipophilic hydrocarbons with DNA has not been documented extensively in the literature, Berry et al. have recently isolated 9-octadecynoic acid from *Schoepfia californica* by bioassay-guided fractionation using a whole cell assay designed to identify DNA binding molecules.[40] Further, Gourévitch et al. have demonstrated DNA binding by a series of steroids by a mechanism that probably includes both ionic and lipophilic elements,[41] and two groups have shown that an oligonucleotide bearing a lipophilic substituent bound with enhanced affinity to the single-stranded complementary oligonucleotide.[42,43] Recently, Letsinger et al. have shown that the self-assembly of oligonucleotides can be controlled by the use of hydrophobic substituents.[44] While most of the studies characterizing the nature of DNA interaction by well-established DNA binding and cleaving agents have focused on modes of association such as intercalation, groove binding via H-bonding, or electrostatic interactions, Ding and Ellestad have reported evidence for a hydrophobic association between DNA and calicheamicin.[45] Further, hydrophobic interactions have been shown to play an important role in the association of certain DNA minor-groove binding agents.[46]

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Experimental Section

3,5-Dimethoxybenzaldehyde was purchased from Lancaster Synthesis. All other organic chemical intermediates were obtained from Aldrich Chemicals, including 1,3-dihydroxy-5-methylbenzene (**1a**), 1,3-dihydroxy-5-pentylbenzene (**1b**), and 2-hydroxy-3-methoxybenzaldehyde. 2,9-Dimethyl-1,10-phenanthroline (neocuproine), *n*-amylamine, all deuterated NMR solvents, and 10% palladium-on-carbon were also obtained from Aldrich. Tris, ethidium bromide, mushroom tyrosinase (1 unit causes an increase of 1×10^{-3} A_{280} units/min at 25 °C in a reaction containing tyrosine as substrate), and bovine liver catalase (1 unit catalyzes the destruction of 1 μmol of H_2O_2/min at 25 °C) were from Sigma Chemicals. ϕX174 Replicative form DNA, plasmid pR322 DNA, and agarose were purchased from Bethesda Research Laboratories, as were restriction endonucleases *Sau*96I and *Bam*HI.

High-field NMR experiments were recorded at 360 MHz on a Nicolet NTC 360 FT NMR spectrometer or on a GE QE-300 NMR spectrometer. Chemical shift values are expressed relative to added tetramethylsilane. Low-resolution chemical ionization and electron impact mass spectra were recorded on a Finnigan MAT 4600 gas chromatograph/mass spectrometer using a direct exposure probe. Methane was employed as a reagent gas with a source pressure of 0.35 Torr and an electron energy of 100 eV. High-resolution mass spectra were obtained from the Washington University Mass Spectrometry Resource using a VG ZAB-SE mass spectrometer. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA.

Syntheses of Compounds 1c,d and 2a−d. The procedures for the syntheses of compounds **1c,d** were closely analogous to those utilized for the syntheses of naturally occurring resorcinols, as described in the preceding paper;[11d] they are provided as supporting information. The procedures for the syntheses of compounds **2a,b** are given below; the preparations of **2c,d** were closely analogous to those of **2b** and are provided as supporting information.

2,3,5-Trimethoxybenzaldehyde (8). To a solution containing 11.0 g (0.28 mol) of NaOH in 250 mL of degassed H_2O was added 15.0 g (0.10 mol) of *o*-vanillin (**7**). The stirred reaction mixture was cooled to 0 °C under N_2 and treated with 27.2 g (0.10 mol) of $K_2S_2O_8$ over a period of 1 h. The dark green reaction mixture was allowed to warm to room temperature and was then stirred at 25 °C for 24 h. The reaction mixture was acidified to pH 2 with concentrated HCl and filtered. The filtrate was heated on a steam bath for 45 min, and the dark red solution was then extracted continuously with ether for 36 h. The ether layer was dried ($MgSO_4$) and concentrated to afford a brown solid. This solid was dissolved in 60 mL of freshly distilled anhydrous acetone, and the solution was treated with 27.6 g (0.2 mol) of anhydrous K_2CO_3, and then dropwise over a period of 15 min with a solution containing 7.2 g (0.06 mol) of dimethyl sulfate in 10 mL of anhydrous acetone. The reaction mixture was heated at reflux for 16 h, and the cooled mixture was filtered. The filtrate was washed successively with 1 N aqueous NaOH, H_2O, and brine. The dried ($MgSO_4$) organic phase was concentrated under diminished pressure to afford a red oil which was applied to a silica gel column (20 × 2.5 cm). The column was washed with 10% ethyl acetate in hexanes. Concentration of the appropriate fractions afforded 2,3,5-trimethoxybenzaldehyde (**8**) as colorless needles with a yield of 1.4 g (7.2%, based on *o*-vanillin (**7**)): mp 62−63 °C; silica gel TLC R_f 0.27 (10% ethyl acetate in hexanes); ^{1}H NMR ($CDCl_3$) δ 3.80 (s, 3 H), 3.86 (s, 3 H), 3.90 (s, 3 H), 6.74 (d, 1 H, J = 2.5 Hz), 6.85 (d, 1 H, J = 2.5 Hz) and 10.39 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 56.00, 56.33, 63.00, 99.59, 107.58, 129.79, 148.07, 154.31, 156.36, 190.13; mass spectrum (chemical ionization) *m/z* 197 $(M + 1)^+$; mass spectrum (electron impact) *m/z* 196.073 ($C_{10}H_{12}O_4$ requires 196.073).

6-(1′-Hydroxypentyl)-1,2,4-trimethoxybenzene (9b). An anhydrous solution of 20 mL of ether under N_2 containing 83 mg (3.4 mmol) of Mg turnings and a small crystal of I_2 was stirred and heated gently during dropwise addition of 466 mg (3.4 mmol) of 1-bromobutane in 10 mL of anhydrous ether. The mixture was heated at reflux for 2 h and then cooled to room temperature and treated dropwise with a solution of 342 mg (1.7 mmol) of 2,3,5-trimethoxybenzaldehyde (**8**) in 10 mL of anhydrous ether. The reaction mixture was heated at reflux for 12 h, then cooled to 25 °C and treated with 10 mL of 0.5 N HCl. The organic layer was separated, and the aqueous layer was extracted with three 10-mL portions of ether. The combined ether layer was washed successively with water, saturated $NaHCO_3$ solution, and brine and then dried ($MgSO_4$) and concentrated under diminished pressure. The resulting yellow oil was purified by chromatography on a silica gel column (20 × 2.5 cm) which was washed with CH_2Cl_2. The appropriate fractions were concentrated under diminished pressure, affording 6-(1′-hydroxypentyl)-1,2,4-trimethoxybenzene (**9b**) as a colorless, viscous oil with a yield of 319 mg (72%): silica gel TLC R_f 0.15 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 7 Hz), 1.30 (m, 4 H), 1.70 (m, 2 H), 2.53 (br s, 1 H, ex D_2O), 3.70 (s, 3 H), 3.74 (s, 3 H), 3.78 (s, 3 H), 4.90 (m, 1 H), 6.37 (d, 1 H, J = 2.5 Hz), 6.46 (d, 1 H, J = 2.5 Hz); ^{13}C NMR ($CDCl_3$) δ 14.44, 23.04, 28.61, 38.52, 55.99, 56.16, 61.50, 70.49, 99.60, 101.92, 138.88, 153.58, 156.66; mass spectrum (electron impact) *m/z* 254 $(M)^+$; mass spectrum (electron impact) *m/z* 254.151 ($C_{14}H_{22}O_4$ requires 254.151).

6-(*trans*-1′-Pentenyl)-1,2,4-trimethoxybenzene (10b). A solution containing 300 mg (1.2 mmol) of 6-(1′-hydroxypentyl)-1,2,4-trimethoxybenzene (**9b**) and 10 mg (58 μmol) of *p*-toluenesulfonic acid in 55 mL of benzene was heated at reflux in an apparatus fitted with a Dean−Stark trap for 14 h. The cooled reaction mixture was washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated under diminished pressure. The oily residue was purified by chromatography on a silica gel column (20 × 2.5 cm); elution with CH_2Cl_2 provided 6-(*trans*-1′-pentenyl)-1,2,4-trimethoxybenzene (**10b**) as a colorless oil with a yield of 235 mg (84%): silica gel TLC R_f 0.65 (CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3 H, J = 7 Hz), 1.50 (m, 2 H), 2.20 (m, 2 H), 3.69 (s, 3 H), 3.73 (s, 3 H), 3.80 (s, 3 H), 6.13 (m, 1 H, J = 16 Hz), 6.32 (d, 1 H, J = 3 Hz), 6.50 (d, 1 H J = 3 Hz), 6.62 (d, 1 H, J = 16 Hz); mass spectrum (electron impact) *m/z* 236 $(M)^+$.

6-(*n*-Pentyl)-1,2,4-trimethoxybenzene (11b). A solution containing 231 mg (0.98 mmol) of 6-(1′-pentenyl)-1,2,4-trimethoxybenzene (**10b**) in 30 mL of ethyl acetate was treated with 25 mg of 10% palladium-on-carbon and stirred under 1 atm of H_2 for 12 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under diminished pressure to afford 6-(*n*-pentyl)-1,2,4-trimethoxybenzene (**11b**) as a colorless oil with a yield of 233 mg (100%): silica gel TLC R_f 0.55 (10% ethyl acetate in hexanes); ^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3 H, J = 7 Hz), 1.30 (m, 4 H), 1.57 (m, 2 H), 2.56 (t, 2 H, J = 8 Hz), 3.66 (s, 3 H), 3.69 (s, 3 H), 3.78 (s, 3 H), 6.22 (d, 1 H, J = 3 Hz), 6.30 (d, 1 H, J = 3 Hz); ^{13}C NMR ($CDCl_3$) δ 14.45, 22.99, 30.51, 30.93, 32.23, 55.88, 56.09, 61.20, 98.10, 105.47, 137.32, 141.56, 153.71, 156.26; mass spectrum (electron impact) *m/z* 238 $(M)^+$. Anal. Calcd for $C_{14}H_{22}O_3$: C, 70.55; H, 9.30. Found: C, 70.13; H, 9.18.

6-(*n*-Pentyl)-1,2,4-trihydroxybenzene (2b). An anhydrous solution containing 66 mg (0.27 mmol) of 6-(*n*-pentyl)-1,2,4-trimethoxybenzene (**11b**) in 10 mL of freshly distilled CH_2Cl_2 under argon was cooled to 0 °C and treated dropwise with 1.5 mL of a 1 M solution of BBr_3 in anhydrous CH_2Cl_2. The reaction mixture was allowed to warm to room temperature and was stirred at 25 °C for 12 h. The reaction was quenched by the addition of 2 mL of H_2O, followed by the addition of 20 mL of brine. The layers were separated, and the organic phase was washed with an additional three 10-mL portions of brine. The dried ($MgSO_4$) organic phase was concentrated under diminished pressure and recrystallized from hexane to afford 6-(*n*-pentyl)-1,2,4-trihydroxybenzene (**2b**) as colorless microcrystals with a yield of 49 mg (92%): mp 88−89 °C; silica gel TLC R_f 0.48 (1:1 hexanes−ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3 H, J = 6.5 Hz), 1.35 (m, 4 H), 1.57 (m, 2 H), 2.53 (t, 2 H, J = 7.5 Hz), 4.30 (s, 1 H, ex D_2O), 4.45 (s, 1 H, ex D_2O), 5.18 (s, 1 H, ex D_2O), 6.18 (d, 1 H, J = 3 Hz), 6.29

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(d, 1 H, J = 3 Hz); ^{13}C NMR ($CDCl_3$) δ 14.44, 22.97, 29.81, 30.27, 32.07, 101.07, 108.18, 128.60, 133.90, 145.13, 152.24; mass spectrum (electron impact) m/z 196 $(M)^+$; mass spectrum (electron impact) m/z 196.109 ($C_{11}H_{16}O_3$ requires 196.109).

2-Hydroxy-3-methoxytoluene (12). A reaction mixture containing 2.0 g (13.2 mmol) of *o*-vanillin (**7**) and 1 g of 10% palladium-on-carbon in 40 mL of ethyl acetate and 10 mL of acetic acid was stirred under a hydrogen atmosphere for 3 days. The reaction mixture was filtered through Celite, and the filtrate was concentrated. The crude product was purified by flash chromatography on silica gel; washing with 10:1 hexane−ethyl acetate afforded **12** as colorless needles with a yield of 1.78 g (98%): mp 37−38 °C; bp 65−70 °C (0.7 mmHg); silica gel TLC R_f 0.64 (4:1 hexanes−ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3 H), 3.83 (s, 3 H), 5.67 (s, 1 H, ex D_2O), 6.76−6.83 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 15.88, 56.42, 108.74, 119.63, 123.68, 124.43, 144.27, 146.76; mass spectrum (chemical ionization) m/z 139 $(M + 1)^+$; mass spectrum (electron impact) m/z 138.068 ($C_8H_{10}O_2$ requires 138.068).

2-Methoxy-6-methyl-1,4-benzoquinone (13). A stirred solution of 1.08 g (7.9 mmol) of KH_2PO_4 in 350 mL of water was treated with 6.38 g (23.8 mmol) of potassium nitrosodisulfonate (Fremy's salt). The resulting solution was treated in one portion with a solution of 1.3 g (9.4 mmol) of 2-hydroxy-3-methoxytoluene (**12**) in 35 mL of ether. The reaction mixture was stirred for 1 h, during which time a yellow precipitate formed. The reaction mixture was extracted with three 100-mL portions of CH_2Cl_2, and the combined organic phase was washed with water and then dried (Na_2SO_4). Concentration of the dried solution afforded 1.0 g of a red solid which provided 2-methoxy-6-methyl-1,4-benzoquinone as fine yellow needles from methanol with a yield of 1.0 g (70%): mp 148.5−149.5 °C; silica gel TLC R_f 0.24 (7:3 hexanes−ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.07 (d, 3 H, J = 1.5 Hz), 3.83 (s, 6 H), 5.86 (d, 1 H, J = 1.5 Hz), 6.53 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 15.99, 56.68, 107.70, 134.22, 144.02, 159.20, 182.75, 187.80; mass spectrum (chemical ionization) m/z 153 $(M + 1)^+$. Anal. Calcd for $C_8H_8O_3$: C, 63.15; H, 5.30. Found: C, 62.92; H, 5.37.

2,5-Dihydroxy-3-methoxytoluene (14). A stirred solution containing 1.0 g (6.6 mmol) of 2-methoxy-6-methyl-1,4-benzoquinone (**13**) in 20 mL of acetone was treated in one portion with a solution of 3.0 g (19.4 mmol) of titanium trichloride in 10 mL of water. The reaction mixture was stirred at room temperature for 10 min and then poured into 50 mL of brine. The reaction mixture was extracted with three 25-mL portions of ether, and the combined organic extract was washed with three 25-mL portions of brine and then dried (Na_2SO_4). Concentration of the organic phase under diminished pressure afforded a white solid that crystallized from $CHCl_3$ to give hydroquinone **14** as colorless needles with a yield of 997 mg (98%): mp 127−128 °C; ^{1}H NMR (acetone-d_6) δ 2.12 (s, 3 H), 3.72 (s, 3 H), 6.18 (d, 1 H, J = 2.5 Hz), 6.32 (d, 1 H, J = 2.5 Hz), 6.50 (s, 1 H, ex D_2O), 7.57 (s, 1 H, ex D_2O); ^{13}C NMR (acetone-d_6) δ 15.52, 55.68, 98.00, 109.00, 124.26, 137.74, 147.74, 150.18; mass spectrum m/z 155 $(M + 1)^+$. Anal. Calcd for $C_8H_{10}O_3$: C, 62.32; H, 6.53. Found: C, 62.23; H, 6.53.

2,3,5-Trimethoxytoluene (11a). A reaction mixture containing 238 mg (1.55 mmol) of hydroquinone **14**, 2.0 g (14.5 mmol) of K_2CO_3, and 500 mg (3.97 mmol) of dimethyl sulfate in 20 mL of acetone was heated at reflux for 12 h. The cooled reaction mixture was filtered, and 50 mL of ether was added to the filtrate. The reaction mixture was washed successively with three 25-mL portions each of 10% aqueous NaOH, water, and brine and then dried (Na_2SO_4). The solution was concentrated, and the residue was purified by flash chromatography on a 30-g silica gel column. Elution with 19:1 hexanes−ethyl acetate provided 6-methyl-1,2,4-trimethoxybenzene (**11a**) as a clear, colorless oil upon concentration of the appropriate fractions. The product crystallized on standing, affording **11a** as tiny, irregular cubes with a yield of 78 mg (28%): mp 26−27 °C; silica gel TLC R_f 0.40 (10:1 hexanes−ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3 H), 3.70 (s, 3 H), 3.73 (s, 3 H), 3.80 (s, 3 H), 6.28 (d, 1 H, J = 3 Hz), 6.35 (d, 1 H, J = 3 Hz); ^{13}C NMR ($CDCl_3$) δ 16.55, 55.90, 56.14, 60.73, 98.23, 106.33, 132.47, 141.83, 153.72, 156.21; mass spectrum m/z 183 $(M + 1)^+$. Anal. Calcd for $C_{10}H_{14}O_3$: C, 65.91; H, 7.74. Found: C, 65.96; H, 7.77.

2,3,5-Trihydroxytoluene (2a). Demethylation of 2,3,5-trimethoxytoluene (75 mg; 0.41 mmol) was carried out using BBr_3 in CH_2Cl_2 as described above for **2b**. The crude product, isolated as a white solid following extractive workup with ethyl acetate, provided 2,3,5-trihydroxytoluene (**2a**) as colorless needles from chloroform with a yield of 38 mg (66%): mp 139−140 °C; silica gel TLC R_f 0.30 (2:1 chloroform−acetone); ^{1}H NMR (acetone-d_6) δ 2.10 (s, 3 H), 6.08 (d, 1 H, J = 2.5 Hz), 6.22 (d, 1 H, J = 2.5 Hz), 6.45 (s, 1 H, ex D_2O), 7.44 (s, 1 H, ex D_2O), 7.83 (s, 1 H, ex D_2O); ^{13}C NMR (D_2O) δ 15.79, 101.52, 108.92, 128.56, 145.95, 149.67, 158.82; mass spectrum (chemical ionization) m/z 141 $(M + 1)^+$. Anal. Calcd for $C_7H_8O_3$: C, 59.99; H, 5.75. Found: C, 59.91; H, 5.72.

General Procedure for the Relaxation of Supercoiled DNA by Di- and Trihydroxyalkylbenzenes. Cleavage of ϕX174 replicative form DNA was carried out in 40-μL reaction mixtures containing 200 ng of DNA and the indicated amounts of di- or trihydroxyalkylbenzene and Cu^{2+} in 50 mM Na cacodylate, pH 7.2. The reactions were run as indicated in the individual figure legends, terminated by the addition of 10 μL of a loading buffer solution (40 mM Tris OAc buffer, pH 7.8, containing 5 mM EDTA, 40% glycerol, 0.4% sodium dodecyl sulfate, and 0.3% bromophenol blue), and applied to 1.2% agarose gels. The gels were run in 40 mM Tris OAc buffer, pH 7.8, containing 1 μg/mL of ethidium bromide at 40 V for 14−16 h.

Treatment of 1,3-Dihydroxy-5-methylbenzene (1a) with *n*-Amylamine. The reaction mixture (100 μL total volume) consisted of an aqueous solution containing 200 μM 1,3-dihydroxy-5-methylbenzene (orcinol, **1a**), 400 μM $CuCl_2$, and 2 mM *n*-amylamine. A stream of O_2 was bubbled through the solution for 5 min under ambient conditions, and then a 10-μL aliquot of the reaction mixture was employed in a DNA cleavage assay (*vide supra*).

Base Treatment of 1,3-Dihydroxy-5-methylbenzene (1a) and 6-Methyl-1,2,4-trihydroxybenzene (2a). A solution containing 160 μM **1a** or **2a** and 160 μM $CuCl_2$ in 50 μL of water was treated with 25 μL of 0.01 N NaOH. A stream of O_2 was bubbled through the reaction mixture for 5 min, after which the solution was neutralized by the addition of 25 μL of 0.01 N HCl. A 10-μL aliquot of the reaction mixture was then used in a DNA cleavage assay (Figure 1).

Base Treatment of 1,3-Dihydroxy-5-methylbenzene (1a) in the Presence and Absence of Cu^{2+}. A solution containing 800 μM **1a**, with or without 1.6 mM Cu^{2+}, in 50 μL of water was treated with 25 μL of 0.1 N NaOH. A stream of air was bubbled through the reaction mixture for 15 min, which was then neutralized by the addition of 25 μL of 0.1 N HCl. A 10-μL aliquot of each solution was then used in a DNA cleavage assay (supporting information, Figure 5).

Base Treatment of 1,3-Dihydroxy-5-methylbenzene (1a) + Cu^{2+} in the Presence and Absence of O_2. A degassed solution containing 800 μM **1a**, with or without 1.6 mM Cu^{2+}, in 50 μL of water was treated anaerobically with a degassed solution of 0.1 N NaOH. The reaction mixture was maintained under anaerobic conditions for 5 min, and then neutralized with 25 μL of 0.1 N HCl. A control reaction containing **1a** and Cu^{2+} was run in parallel under aerobic conditions. A 10-μL aliquot of each solution was used in a DNA cleavage reaction, which was run under ambient conditions.

DNA Strand Scission by 6-Methyl-1,2,4-trihydroxybenzene (2a) + Cu^{2+} in the Presence and Absence of O_2. A degassed solution containing 10 μM **2a**, 20 μM Cu^{2+}, and 200 ng of DNA in 40 μL of buffer was maintained under an argon atmosphere at 25 °C for 20 min. A control reaction containing **2a** + Cu^{2+} was run in parallel under ambient conditions.

DNA Strand Scission by 1,3-Dihydroxy-5-methylbenzene (1a) and 6-Methyl-1,2,4-trihydroxybenzene (2a) in the Presence and Absence of Neocuproine. The reaction mixture contained 100 μM **1a** or **2a**, 100 μM Cu^{2+}, and 200 ng of DNA in 40 μL of 50 mM Na cacodylate, pH 7.2, with or without 200 μM 2,9-dimethyl-1,10-phenanthroline (neocuproine). The reaction mixtures were incubated at 25 °C for 30 min, then analyzed by agarose gel electrophoresis.

DNA Strand Scission by 6-Methyl-1,2,4-trihydroxybenzene (2a) after Treatment with DDQ. The reaction mixture contained 80 μM **2a** and 800 μM 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in 20 μL of H_2O, which were incubated at 25 °C for 30 min and then treated with 5 μL of 240 μM Cu^{2+} and 200 ng of DNA in 15 μL of Na cacodylate buffer. The combined solution (40 μL total volume, containing **2a**, Cu^{2+}, and DDQ at concentrations of 40, 30, and 200

μM, respectively) was maintained at 25 °C for 30 min and then analyzed by agarose gel electrophoresis.

DNA Strand Scission by 6-Methyl-1,2,4-trihydroxybenzene (2a) in the Presence and Absence of DMSO. The reaction mixture contained 25 μM **2a**, 100 μM Cu^{2+}, and 200 ng of DNA in 40 μL of 50 mM Na cacodylate, pH 7.2, with or without 10% dimethyl sulfoxide (DMSO). The reaction mixtures were incubated at 25 °C for 15 min, then analyzed by agarose gel electrophoresis.

DNA Strand Scission by 6-Methyl-1,2,4-trihydroxybenzene (2a) in the Presence of Catalase and Tyrosinase. The reaction mixture contained 25 μM **2a**, 100 μM Cu^{2+}, and 200 ng of DNA in 40 μL of 50 mM Na cacodylate, pH 7.2, with either 0.1 unit of catalase or 5 units of tyrosinase. The reactants were added simultaneously in all reactions and maintained at 25 °C for 15 min, except for those incubations involving denatured tyrosinase in which **2a** + tyrosinase were incubated for 5 min prior to the addition of Cu^{2+} and DNA. The reaction mixtures were analyzed by agarose gel electrophoresis.

Degradation of ^{32}P End Labeled DNA Duplexes by Di- and Trihydroxyalkylbenzenes. In a typical experiment, a 149-base pair (bp) DNA fragment was obtained from plasmid pBR322 DNA by treatment with restriction endonucleases and was then 5′-^{32}P end labeled, essentially as described.[47] Reactions were carried out in 40 μL (total volume) of 50 mM Na cacodylate, pH 7.0, containing ^{32}P-labeled DNA duplex (3×10^4 dpm), 0.1 mM calf thymus DNA, 20 μM Cu^{2+}, and 50 μM, 100 μM, 200 μM, or 2 mM 6-(*n*-pentyl)-1,2,4-trihydroxybenzene (**2b**). The reaction mixture was maintained at 25 °C for 1 or 2 h, then quenched by the addition of 5 μL of 50 mM NaOAc, pH 5.5, containing 10 mM $MgCl_2$ and 2 μL of a solution containing 5 mM calf thymus DNA. The DNA was recovered by ethanol precipitation, then analyzed by electrophoresis on a 10% denaturing polyacrylamide gel.

Maxam–Gilbert sequencing[48] and Fe(II)·MPE cleavage[34] lanes were run as described.

Degradation of d(CGCT$_3$A$_3$GCG) by 6-Methyl-1,2,4-trihydroxybenzene (2a). An aqueous solution (100 μL total volume) containing 1 mM 5′-d(CGCT$_3$A$_3$GCG)-3′ (DNA nucleotide concentration), 0.5 mM 6-methyl-1,2,4-trihydroxybenzene, and 1 mM $CuCl_2$ was maintained at 25 °C for 1 h. A 20-μL aliquot was then analyzed by C_{18} reverse phase HPLC. Elution with 0.1 M NH_4 OAc, pH 6.8, at a flow rate of 1.6 mL/min afforded peaks of material that co-migrated with authentic cytosine (1.7 min), guanine (4.3 min), thymine (5.7 min), and adenine (10.5 min).

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Supporting Information Available: Experimental descriptions of the methods for synthesizing **1c,d**, and **2c,d** and nine figures illustrating the cleavage of DNA by di- and trihydroxyalkylbenzenes under different experimental conditions (23 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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