represented only a small portion of the total weights of these fractions.

Quantitative determinations of most of the reducing sugars were carried out by a paper chromatographic-colorimetric method developed in this laboratory.[33] The cellobiose, -triose, and -tetraose fractions obtained from irradiated and blank cellopentaose samples were first hydrolyzed, using 4% aq. sulfuric acid at 100° for 6.5 hr., and the quantities of D-glucose in the processed hydrolyzates were determined by the above method.[33] The 3-β-D-glucopyranosyl-D-arabinose fractions were not large enough for this analysis, and no 3-β-cellobiosyl-D-arabinose was detected in the appropriate fractions. The hydrolyzate of the cellotetraose fraction from irradiated cellopentaose contained some D-arabinose, the quantity of which was also determined. This D-arabinose was assumed to be derived from 3-β-cellotriosyl-D-arabinose, another degradation product, which was present in the cellotetraose fraction as a contaminant.

The quantities of these oligosaccharides were then calculated, after correction for the blanks, from the quantities of the monosaccharides in the hydrolyzates. The quantity of 3-β-cellotriosyl-D-arabinose arrived at is almost certainly too low, some of that sugar having no doubt been located on the sheet immediately below the excised area of cellotetraose.

The quantities of methyl β-D-glucopyranoside and D-glucitol were finally estimated by visual comparison. The appropriate fractions from irradiated and blank methyl β-cellobioside, cellobiitol, and cellopentaitol, and solutions of authentic methyl β-D-glucopyranoside and D-glucitol in a suitable range of concentrations were, for this purpose, chromatographed side by side on the same strips of paper. Developer A was used for methyl β-D-glucopyranoside, developer E for D-glucitol, and spray reagent β for both.

(33) J. E. Jeffery, E. V. Partlow, and W. J. Polglase, *Anal. Chem.*, **32**, 1774 (1960).

Acknowledgment. The authors thank Prof. M. L. Wolfrom for the gift of cellobiitol seed crystals; Prof. F. Smith for an authentic specimen of methyl β-glucopyranoside; and Mr. E. V. Partlow for the synthesis of the same compound.

SHELTON, WASH.

[CONTRIBUTION NO. 55 FROM THE OLYMPIC RESEARCH DIVISION OF RAYONIER INC.]

Lignin Model Compounds. Nitric Acid Oxidation of 4-Methylguaiacol[1]

IGOR SOBOLEV[1a]

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4-Methylguaiacol (I) reacts with dilute aqueous nitric acid giving rise to the following sequence of intermediates: I ⟶ 4-methyl-6-nitrosoguaiacol (II) ⟶ 4-methyl-6-nitroguaiacol (III) ⟶ 5-methyl-3-nitropyrocatechol (IV) ⟶ 2-hydroxy-5-methyl-3-nitro-1,4-benzoquinone (V). End products include nitrous oxide, nitrogen, nitric oxide, nitrogen dioxide, carbon monoxide, carbon dioxide, and oxalic acid. Nitrosonium (NO^+) ion mechanisms for the nitration of I and demethylation of III are proposed to account for the observed catalysis by nitrous acid. Similar reactions are believed to occur in lignin oxidations.

Oxidation with aqueous nitric acid is one of the oldest yet least understood reactions for degrading lignin. Only the simplest products have been identified so far: monomeric phenols and nitrophenols, oxalic, acetic and formic acids, carbon monoxide, and carbon dioxide. Reported reduction products from nitric acid include nitrogen, nitrous oxide, nitric oxide, nitrogen dioxide, and hydrogen cyanide. Some cleavage of aromatic methoxyl groups occurs also, and nitrous acid appears to be a required catalyst for the oxidation.[2] Oxidations of model compounds[3,4] have offered little new evidence concerning reaction mechanisms. In organic solvents, nitration of lignins involves some electrophilic displacement of aliphatic side chains by nitro groups.[5] Structures undergoing this displacement are chiefly 4-substituted guaiacyl end units containing benzyl alcohol and ether groups. To what extent displacement occurs in oxidations with aqueous nitric acid has not been established. The results of the present model compound study, applicable to portions of lignin, are believed to be of value primarily in indicating the role of nitrous acid in oxidations with nitric acid.

4-Methylguaiacol (I), a simplified lignin model with no side-chain oxygen, was oxidized with 2.6*N* "pure"[6] nitric acid at 70°. In a two-hour reaction, nearly all the compound was oxidized to oxalic acid, other water-soluble products, and to the gases listed in Table I. From the evidence presented

(1) Presented at the 138th National Meeting of the American Chemical Society, New York, N. Y., September 1960. (a) Present address: Shell Development Co., Emeryville, Calif.

(2) F. E. Brauns, *The Chemistry of Lignin*, Academic Press, New York, 1952; L. L. Sergeeva, A. A. Chuksanova, and N. N. Shorygina, *Izvest. Akad. Nauk SSSR, Otdel. Khim. Nauk*, No. 5, 653 (1957); *Chem. Abstr.*, **51**, 14261b (1957); V. M. Nikitin and A. E. Sosnin, *Trudy Leningrad. Lesotekh. Akad.*, No. 75, 67 (1956); *Chem. Abstr.*, **53**, 10744b (1959).

(3) O. Routala and J. Sevon, *Cellulosechem.*, **7**, 113 (1926).

(4) K. Ley and E. Müller, *Chem. Ber.*, **89**, 1402 (1956).

(5) C. Gustafsson and L. Andersen, *Paperi ja Puu*, **37**, 1 (1955); A. A. Chuksanova, L. L. Sergeeva, and N. N. Shorygina, *Izvest. Akad. Nauk SSSR, Otdel. Khim. Nauk*, No. 12, 2219 (1959).

(6) In this paper, "pure" nitric acid refers to solutions prepared from 70% reagent grade acid without addition of nitrous acid. "Nitrous acid" denotes all those chemical species determined as nitrous acid by the analytical method of Nelson, Kurtz, and Bray [*Anal. Chem.*, **26**, 1081 (1954)], *i.e.*, by colorimetric estimation of an azo dye formed in aqueous acetic acid.

TABLE I

OXIDATIONS WITH AQUEOUS NITRIC ACID[a]

Compound	4-Methyl-guaiacol (I)	4-Methyl-6-nitro-guaiacol (III)[b]			Quinone V[c]
Initial HNO_3, N	2.63	2.60	2.50	2.50	2.50[d]
Initial HNO_3, m./m. starting compound	20	19.5	19.5	19.5	18.5
Time, hr.	2.0	2.0	2.5	2.1	2.0
Temperature	68–72	68–72	68–73	75–77	75–77
HNO_3 consumed, m./m. starting compound	5.5	4.0	4.2	4.5	2.9
Oxalic acid formed, m./m.	0.87	0.31	0.55	0.58	0.42
CO formed, m./m.	0.26	0.19	0.21	0.39	0.17
CO_2 formed, m./m.	1.81	1.58	1.73	2.22	2.07
N_2O formed, m./m.	0.80	0.78	0.82	1.05	1.07
N_2 formed, m./m.	0.13	0.08	0.12	0.13	0.14
NO + NO_2 formed, m./m.	0.29	0.14	0.31	0.43	<0.03
Organic acids formed, equivs./mole starting compound	3.5	3.0	3.7	3.8	3.1
Insoluble residue, % by wt. of starting compound	4.5	0	0	0	0

[a] All oxidations were carried out with 7–10-g. quantities of compounds by the procedure described for I. [b] M.p. 79–80°. [c] M.p. 116–118°. [d] Contained added sodium nitrite in 0.001M concentration.

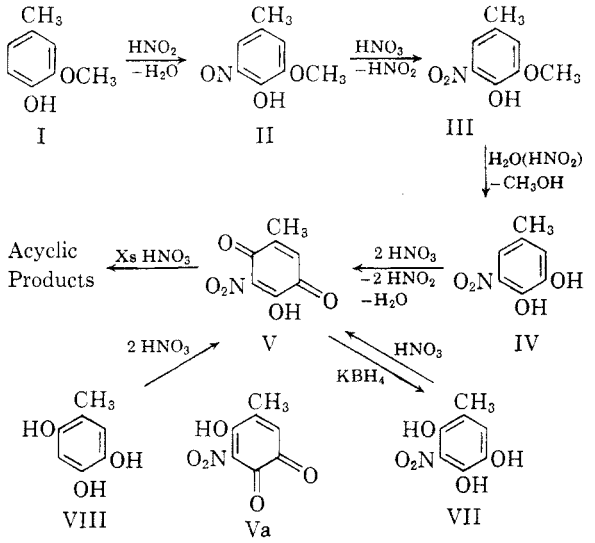


Figure 1

below, it was concluded that an appreciable portion of the model compound I underwent the reaction sequence I → II → III → IV → V → acyclic products.

The formation of 4-methyl-6-nitrosoguaiacol (II) as the first intermediate was inferred from the fact that the first detectable reaction of I with nitric acid was nitration *via* nitrosonium (NO^+) ion. When compound I was heated with dilute nitric acid, and the reaction was quenched at an early stage, 4-methyl-6-nitroguaiacol[7,8] was isolated in crystalline form. The product pattern and nitric acid consumption found upon further oxidation of III with nitric acid (Table I) indicated that it was a true intermediate. Catalysis of the initial reaction of I by nitrous acid and inhibition by urea suggested that nitration proceeded by the special nitration mechanism described by Ingold and coworkers.[9] According to this mechanism, outlined by reactions 1–5, initial attack on I is by nitrosonium ion, which is derived from molecular nitrous

$$HNO_2 + HNO_3 \rightleftharpoons H_2NO_2^+ + NO_3^- \quad (1)$$

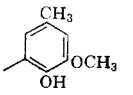
$$H_2NO_2^+ \rightleftharpoons NO^+ + H_2O \quad (2)$$

$$NO^+ + NO_3^- \rightleftharpoons N_2O_4 \quad (3)$$

$$ArH + NO^+ \longrightarrow ArNO + H^+ \quad (4)$$

$$ArNO + HNO_3 \longrightarrow ArNO_2 + HNO_2 \quad (5)$$

Ar =



acid or other nitrosonium ion "carriers."[10] Compound II, like other nitrosophenols,[9] was apparently rapidly oxidized to III and thus escaped direct detection. Concurrent nitration by nitronium (NO_2^+) ion appeared to be negligible under these conditions. The reaction of I with aqueous nitric acid was autocatalytic; this was due to the net production of nitrous acid in a later step of the reaction sequence (*vide infra*). The nitrosonium ion–catalyzed nitration of I was more easily controlled in aqueous acetic acid or chloroform–acetic acid. Using these solvents, compound III could be prepared in high yields by treating I with an equimolar amount of nitric acid.

The next step in the reaction sequence was studied by allowing III to react with limited amounts of nitric acid in various solvents. When the reaction was run in organic solvents, 5-methyl-3-

(7) M. Oberlin, *Arch. Pharm.*, **263**, 641 (1925).

(8) J. M. Gulland and R. Robinson, *J. Chem. Soc.*, 1971 (1926).

(9) C. A. Bunton, E. D. Hughes, C. K. Ingold, D. I. H. Jacobs, M. H. Jones, G. J. Minkoff, and R. I. Reed, *J. Chem. Soc.*, 2628 (1950).

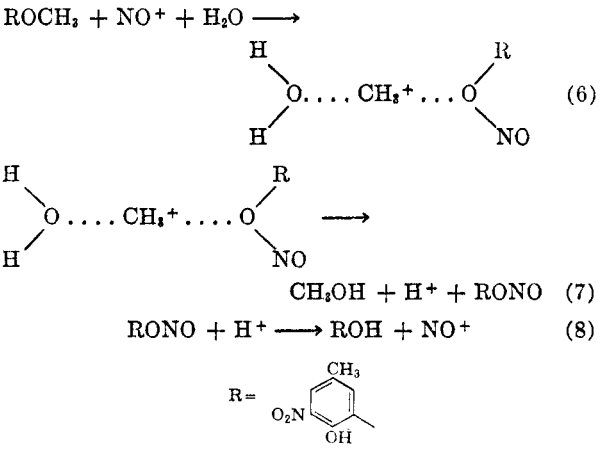
(10) T. A. Turney and G. A. Wright, *Chem. Revs.*, **59**, 497 (1959).

nitropyrocatechol[11] (IV) and a second product, later identified as 2-hydroxy-5-methyl-3-nitro-1,4-benzoquinone (V), were isolated in crystalline form as the only products. Compound IV reacted smoothly with nitric acid to give the quinone V in good yield. In solvents containing appreciable amounts of water, compound IV was not detected, and V was the only product. Reaction of III with nitric acid was also catalyzed by nitrous acid, indicating that nitrosonium ion was required for methoxyl cleavage. Simple acid hydrolysis was not involved, since other mineral acids (hydrochloric acid, sulfuric acid) of comparable concentrations had no effect on III.

The intermediate quinone V, evidently arising by oxidation of IV, could be prepared in good yields by treatment of III with nitric acid in a 1:2 ratio using aqueous acetic acid or chloroform–acetic acid as solvents. The structure of the quinone was established on the basis of absorption spectra,[12] elemental and functional group analyses, and alternate synthesis from 2,4,5-trihydroxytoluene (VIII). Neither the quinone nor its presumably less stable tautomer Va have been reported in the literature. The quinone was also identified as one of the products of partial oxidation of I with hot aqueous nitric acid. Upon further oxidation with aqueous nitric acid containing added nitrous acid, the quinone yielded the end products and consumed nitric acid in quantities expected of this intermediate.

Evidence for the mechanism of methoxyl cleavage was obtained from the products of the demethylation and oxidation of III to V. Two moles of nitric acid was consumed, and nitrous acid was produced at the same rate as V (Table II). The cleaved methyl group was recovered as methyl nitrite. Water was found as the remaining product from oxidations in organic solvents. Control experiments showed that any methanol produced in the reaction would have been rapidly esterified by nitrous acid and isolated as methyl nitrite. Since water was always present in greater concentration than molecular nitrous acid, and since it appears to be more strongly nucleophilic,[10] it is reasonable to expect that water was a reactant in methoxyl cleavage. Assuming therefore methanol as a primary demethylation product, the results are consistent with the termolecular displacement mechanism indicated by reactions 6–8.

Formation of a "free" methyl carbonium ion in an S_N1 reaction as originally proposed by Ingold and co-workers for the demethylation of anisoles[9] is unlikely, since no methyl acetate was detected when



the reaction was run in nearly anhydrous acetic acid.

Further oxidation of the quinone V with hot aqueous nitric acid proceeded satisfactorily only in the presence of added nitrous acid. In oxidations of I or III, where the quinone arose as an intermediate, nitrous acid was always available from the oxidation of IV to V. With the quinone as the starting compound, treatment with "pure" nitric acid (with no nitrous acid added) unexpectedly resulted in partial reduction of the quinone to 3-nitro-2,4,5-trihydroxytoluene (VII). This compound could be prepared in high yield by reduction of the quinone with borohydride. It was also obtained in 27% yield when pure aqueous solutions of the quinone were allowed to stand at room temperature. Compound VII, a relatively weak reducing agent, has not been reported in the literature.

In oxidations of lignin with dilute nitric acid, a sequence of reactions analogous to that for 4-methylguaiacol would be expected for guaiacyl end groups not undergoing chain displacement. The results indicate that nitrous acid is a key reactant in the oxidation of such end groups, facilitating nitration, methoxyl cleavage, and oxidation of quinoid intermediates to acyclic products. These functions of nitrous acid are probably not restricted to guaiacyl end groups, but extend to other structural units of lignin as well. Formation of nitrous acid undoubtedly also accounts for the autocatalytic characteristics of many lignin oxidations.

EXPERIMENTAL[13]

Materials. Du Pont reagent grade 70% nitric acid, Baker 97% fuming nitric acid, and petroleum ether, b.p. 30–60°, were used. Eastman practical grade 2-methoxy-4-methylphenol (4-methylguaiacol), shown to be at least 99% pure

(11) H. Cousin, *Ann. chim.*, **13**, 557 (1898); J. M. Gulland and R. Robinson, *J. Chem. Soc.*, 1976 (1926).

(12) L. F. Fieser, *J. Am. Chem. Soc.*, **50**, 439, 465 (1928); W. Flaig, J. C. Salfeld, and E. Baume, *Ann.*, **618**, 117 (1958); J. Pošpisil and V. Eitel, *Coll. Czechoslov. Chem. Commun.*, **24**, 729 (1959); J. Salfeld, *Chem. Ber.*, **93**, 737 (1960).

(13) All melting points were observed on a Fisher-Johns hot stage and are corrected. Elemental and some methoxyl analyses were performed by Berkeley Analytical Laboratory, Berkeley, Calif. Infrared spectra were determined with a Perkin-Elmer Model 21 instrument, and ultraviolet spectra with a Cary Model 11 MS recording spectrophotometer, using 95% ethanol or aqueous solutions (*p*H 3, dilute hydrochloric acid; *p*H 6, phosphate buffer, *p*H 12, borate buffer) as solvents.

by gas chromatography, was employed without further purification.

Analytical methods. Nitric and nitrous acids were determined colorimetrically.[6] Oxalic acid was determined gravimetrically by precipitation with calcium acetate from solutions previously adjusted to *p*H 3 with ammonium hydroxide. The purity of the calcium salt was checked by permanganate titration. Gas chromatography was carried out with a Perkin-Elmer Model 154C recording Vapor Fractometer at room temperature. The gas stream from oxidations with hot aqueous nitric acid was analyzed using a 2-meter "J" (silica gel) column and helium as the carrier gas. Gas compositions were calculated from areas[14] under recorded peaks of chromatograms. In separations by descending paper chromatography, Whatman No. 1 and Whatman Seed Test Paper were employed with the following solvents: (A), 1-propanol-water–ammonium hydroxide, 80:16:4 parts by volume; (B), 3% aqueous acetic acid; (C), 1-butanol–acetic acid–water, 4:1:5 parts by volume, organic phase.

Oxidation of 4-methylguaiacol (I) *with hot aqueous nitric acid.* The apparatus, consisting of a three necked 1-l. flask equipped with a thermometer and a gas inlet tube, was connected through a water-cooled reflux condenser to the inlet of the gas sampling valve of the gas chromatograph. Gas flow rates were measured by water displacement, using water previously saturated with gaseous oxidation products. In the typical experiment listed in Table I, 600 ml. of 2.63*N* "pure" aqueous nitric acid and 10 ml. of 4-methylguaiacol (0.079 mole) were rapidly heated with magnetic stirring to 70 ± 2° in an atmosphere of helium and maintained at that temperature for 2 hr. Gas evolution diminished from an initial rate of approximately 150 ml./min. to 10 ml./min. at the end of 2 hr. One-milliliter samples of the gas stream were diverted for gas chromatography at 20-min. intervals. Components of the gas mixture were identified by comparison of their elution times with those of authentic compounds.[15] The elution time for the nitrogen dioxide peak was somewhat variable and appeared to depend on the moisture content of the column; in addition, both nitric oxide and nitrogen dioxide exhibited trailing peaks with some overlapping. In calculations of gas composition the two gases were therefore treated as one. The two major constituents of the gas stream, carbon dioxide and nitrous oxide, from four successive samples were collected in a liquid nitrogen trap after chromatography, transferred to a 7.5-cm. gas cell with sodium chloride windows, and identified by comparison of their infrared spectra with identically chromatographed authentic gases. At the end of the oxidation, 0.46 g. of a brown solid residue was filtered from the solution. This residue was not investigated. Paper chromatography of the product solution indicated that phenols and quinones were absent, since no colors developed upon spraying of the irrigated chromatograms with ferric ferricyanide solution or 2,4-dinitrophenylhydrazine in 2*N* hydrochloric acid.

Isolation of products from partial oxidation of I *with aqueous nitric acid.* A suspension of 5.0 ml. of I (0.029 mole) in 25 ml. of 2.6*N* "pure" aqueous nitric acid was rapidly heated with stirring to a temperature of 75°. About 5 sec. after the onset of the oxidation, the mixture was diluted with cold water and extracted with chloroform. Drying and evaporation of the extract gave 5.36 g. of a brown oil. Paper chromatography revealed the presence of a major colored product (R_f 0.65, solvent A) displaying the color reactions of a phenol. Preparative chromatography on Whatman Seed Test Paper using solvent A, followed by elution with methanol, yielded 0.155 g. brick red needles, m.p. 72–75°. Further purification with charcoal in ether raised the m.p. to 78.0–79.5°. The infrared spectrum (chloroform solution) exhibited bands at 1550 (NO_2), 1296 (NO_2), and 3230 cm.$^{-1}$ (OH). The ultraviolet absorption spectra exhibited the maxima listed for compound III below. A mixture melting point with authentic III (prepared as described below) was undepressed at 78.0–79.5°.

In a second similar oxidation, the reaction was allowed to proceed for about 15 sec. before quenching by addition of ice. Paper chromatography of the chloroform extract disclosed the presence of a yellow product (R_f 0.83, solvent B) giving the color reactions of a nonphenolic carbonyl compound. One fifth of the chloroform extract was chromatographed on a sheet of Whatman Seed Test Paper using solvent B. While still wet, the zone containing the carbonyl compound was rapidly eluted with water (150 ml.). The ultraviolet spectrum of the filtered and diluted (1:20) eluate exhibited maxima at 270 mμ (A = 1.20) and 330 mμ (A = 0.39) in a *p*H 6 buffer solution; the position of the maxima remained essentially unchanged when the spectrum was recorded in *p*H 3 or *p*H 12 buffers. All these spectra were run against dilute aqueous acetic acid of the same concentration as the eluate. Using the extinction coefficients obtained later for pure V, a 3% yield (based on I) of compound V was calculated from the absorbance of the eluate. Efforts to isolate the carbonyl compound from the aqueous eluate were unsuccessful.

4-Methyl-6-nitroguaiacol (III). To a solution of 10.0 ml. (0.079 mole) of 4-methylguaiacol in 200 ml. of chloroform was added 31 ml. of 2.57*N* fuming nitric acid (0.080 mole) in acetic acid. After 1 min., the solution was extracted with water and evaporated under reduced pressure. Recrystallization of the residue from hot aqueous methanol gave 9.61 g. (66.5%) brick red needles, m.p. 79–80°. Three more recrystallizations raised the m.p. to 80.0–80.5° (lit., m.p. 81–82° uncorr.,[7] 80°[8]).

Anal. Calcd. for $C_8H_9NO_4$: C, 52.46; H, 4.95; N, 7.65. Found: C, 52.72, 52.92; H, 5.15, 5.03; N, 7.62, 6.62.

Ultraviolet absorption (λ_{max}, mμ; log ε): 95% ethanol, 224 (4.12), 294 (3.72), 373 (3.28); *p*H 6 buffer, 222 (4.10), 300 (3.75), 382 (3.24); *p*H 12 buffer, 229 (4.08), 311 (3.59). The infrared spectrum (chloroform) exhibited bands at 3230, 3040, 1550, 1328, 1270, 1245, and 1140 cm.$^{-1}$, and was identical with the spectrum of the sample of III isolated from the partial oxidation of I.

The acetate was prepared with acetic anhydride in the presence of a trace of sulfuric acid. Recrystallization from chloroform–petroleum ether gave a 67% yield of cream-colored needles, m.p. 90–91° (lit.,[7] m.p. 89–90° uncorr.). In a hydrogenation of 25 mg. III in 95% ethanol, using 10% palladium on charcoal as catalyst, 3.03 moles of hydrogen/mole III was absorbed at room temperature. Treatment of III with methylmagnesium bromide in a Zerewitinoff determination yielded 1.09 moles of methane/mole III.

2-Hydroxy-5-methyl-3-nitro-1,4-benzoquinone (V) *from* III. A solution of 1.83 g. of III (0.010 mole) in 100 ml. of chloroform was treated with 10 ml. of 2.20*N* fuming nitric acid in acetic acid at room temperature. After 30 min., the solvent was rapidly evaporated under vacuum at room temperature. Recrystallization of the residue from ether–petroleum ether afforded the product in a yield of 1.33 g. (73% of theory) as yellow crystals melting at 109–112° dec. Three more recrystallizations from ether–petroleum ether gave yellow needles, m.p. 118.5–119.5 dec. Somewhat lower yields of V were obtained (50–60%) when the fuming nitric acid was added in methylene chloride solution rather than in acetic acid. Yields in either solvent mixture were not increased by using a 3:1 ratio of nitric acid to III; with a 1:1 ratio, yields were considerably lower.

Anal. Calcd. for $C_7H_5NO_5$: C, 45.91; H, 2.75; N, 7.65; OCH_3, none. Found: C, 45.93; H, 2.79; N, 8.36; OCH_3, 0.56.

Ultraviolet absorption (λ_{max}, mμ; log ε): 95% ethanol, 266 (4.07), 330 (3.44); *p*H 3 aqueous acid, 269 (4.13), 331 (3.64); *p*H 6 buffer, 270 (4.15), 330 (3.66); *p*H 12 buffer, 269 (4.10); 329 (3.69). The infrared spectrum in chloroform exhibited bands at 3390, 3050, 1680, 1624, 1548, 1400, 1350, and 1300 cm.$^{-1}$.

(14) Areas under recorded peaks were determined using an electromechanical integrator constructed by Mr. M. W. Folsom of this laboratory.

(15) Obtained from The Matheson Co., Inc., Newark, Calif.

Paper chromatographic behavior with solvents B and C was identical with that of the carbonyl compound detected after the partial oxidation of I. Hydrogenation of V over palladium on charcoal resulted in an uptake of 4.0 moles of hydrogen/mole V, and 0.99 mole of methane/mole V was formed in a Zerewitinoff determination. The quinone was highly soluble in water and in polar organic solvents, but nearly insoluble in petroleum ether. It decomposed within a day in aqueous solution (even in the dark), more slowly in organic solvents. Partial decomposition was also noted within two weeks during storage of the crystalline compound in sealed containers in the dark.

3-Nitro-2,4,5-trihydroxytoluene (VII). To a solution of 0.800 g. of the quinone V in 50 ml. of methanol, a total of 4 g. of potassium borohydride was added over a period of 1 hr. The product mixture was acidified with aqueous hydrochloric acid and extracted with chloroform. The residue obtained after drying and evaporation of the extract was dissolved in benzene, filtered, and evaporated, giving 0.49 g. (61%) of reddish black crystals, m.p. 120–124°. Recrystallization from hot aqueous methanol yielded reddish black needles, m.p. 125–127°. Sublimation of the compound at temperatures above 100° in the presence of air did not affect its color or melting point. A Zerewitinoff determination yielded 3.16 moles of methane/mole VII.

Anal. Calcd. for $C_7H_7NO_5$: C, 45.41; H, 3.81; N, 7.57; OCH_3, none. Found: C, 45.32, 45.29; H, 3.94, 4.02; N, 7.11, 7.26; OCH_3, 0.42.

Ultraviolet absorption (λ_{max}, mμ; log ε): 95% ethanol, 266 (3.76), 326 (3.85); aqueous acid, *p*H 3, 262 (3.70), 326 (3.86); *p*H 6 buffer, 269 (4.13), 332 (3.63). The infrared spectrum in chloroform exhibited bands at 3580, 3270, 3040, 1630, 1552, 1395, 1375, 1190 and 1124 cm.$^{-1}$.

Quinone V *from 2,4,5-trihydroxytoluene* (VIII). A solution of 2.198 g. of 2,4,5-triacetoxytoluene,[16] m.p. 112–114°, in 100 ml. of methanol containing dry hydrogen chloride was refluxed under nitrogen for 1 hr. The solution was concentrated under reduced pressure to about 10 ml.; any water present was removed by codistillation with benzene. Extraction of the residue with hot benzene, followed by cooling, gave the product (VIII) as white crystals, m.p. 126–130° (lit.,[16] m.p. 131–132°) in a yield of 0.335 g. (28%).

A suspension of 0.330 g. of VIII (0.00236 mole) in 25 ml. of chloroform was treated with 2 ml. of 2.9*N* fuming nitric acid in acetic acid. The suspended material assumed a transient purplish black color (probably due to formation of VII) and dissolved within a few seconds, giving an orange, slightly cloudy solution. Removal of the solvent and purification of the residue gave yellow crystals, m.p. 116–118° dec. in a yield of 0.217 g. (50%). After two more recrystallizations from ether–petroleum ether, the product (V) melted at 118–119.0° dec. It was found identical with the sample of V prepared from III by mixture melting point determination and comparison of infrared spectra.

4,6-Di-tert-butyl-3-nitro-1,2-benzoquinone. This compound was needed for a comparison of ultraviolet spectra. Preparation according to the published procedure,[4] followed by two recrystallizations from cyclohexane, gave the product as red needles, m.p. 168.5–170.0° (lit.,[4] m.p. 168.0–169.5°) in a yield of 24%. Its infrared absorption spectrum was in good agreement with that reported in the literature.[4] The ultraviolet spectrum in 95% ethanol exhibited maxima at 252 mμ (log ε 3.97) and 362 mμ (log ε 3.79).

Quinone V *from 3-nitro-2,4,5-trihydroxytoluene* (VII). A solution of 0.231 g. of VII (0.00125 mole) in 15 ml. of chloroform was treated with 0.50 ml. of 2.5*N* fuming nitric acid in acetic acid. After 1 min., the orange, slightly cloudy product solution was placed under vacuum and evaporated to dryness without any attempt to quench the reaction. The yellow, crystalline residue was recrystallized once from ether–petroleum ether, giving 0.212 g. (92.6%) of yellow needles, m.p. 118–119°, undepressed upon admixture with V prepared from III.

5-Methyl-3-nitropyrocatechol (IV). A solution of 1.83 g. of 4-methyl-6-nitroguaiacol (III) (0.010 mole) in 100 ml. of chloroform was treated with 5 ml. of 2.0*N* fuming nitric acid in methylene chloride. After 5 min. the solution was rapidly evaporated at reduced pressure. Paper chromatography of the residue disclosed the presence of the starting material III, the quinone V, and a new, yellow product (R_f 0.50, solvent B) giving the color reactions of a phenol. Column chromatography of the dried residue with 2.5% methanolic benzene on silicic acid separated the mixture into two bands, the faster-moving of which consisted of a mixture of III and the new product. Evaporation of the eluate containing this band and recrystallization of the residue from petroleum ether gave a mixture of 0.515 g. (30.6% of theory) of yellow crystals of IV, m.p. 75–80°, and a small amount of red crystals of the starting material, m.p. 78–80°. Three more recrystallizations from petroleum ether, combined with manual separation of crystals of III, yielded 0.308 g. yellow needles, m.p. 80.5–82.0° (lit.,[11] m.p. 82–83°).

Anal. Calcd. for $C_7H_7NO_4$: C, 49.71; H, 4.17; N, 8.28; OCH_3, none. Found: C, 49.19, 49.34; H, 4.15, 4.32; N, 8.05, 8.24; OCH_3, 1.4.

Ultraviolet absorption (λ_{max}, mμ; log ε); 95% ethanol, 222 (4.05), 304 (3.80); *p*H 6 buffer, 217 (4.04), 305 (3.78). The infrared spectrum in chloroform exhibited bands at 3580, 3250, 3050, 2920, 1550, 1345, 1290, and 1238 cm.$^{-1}$.

Quinone V *from* IV. A solution of 0.150 g. of IV (0.00088 mole) in 20 ml. of chloroform was treated with 1.0 ml. of 1.7*N* fuming nitric acid in acetic acid. After 30 min. at room temperature, the solvent was removed under reduced pressure. Recrystallization of the residue from ether–petroleum ether gave 0.096 g. of yellow crystals, m.p. 113–116° (59%). After further recrystallizations the m.p. was 118–119°, undepressed after admixture of V prepared directly from III. The infrared spectra of both materials were identical.

Stability of quinone V *toward nitric acid in an organic solvent.* To a solution of 0.230 g. of V (0.00125 mole) in 30 ml. of chloroform was added 1 ml. of 2.5*N* fuming nitric acid in acetic acid. After 20 hr. at room temperature, the starting compound was recovered after one recrystallization from ether–petroleum ether as yellow crystals, m.p. 118–119°, in 79% yield.

Partial reduction of quinone V *in aqueous solution.* A solution of 0.171 g. of V (0.00093 mole) in 25 ml. of deionized water was stored in the dark at room temperature. After 20 hr., the solution was dark purple and contained a crystalline precipitate. The crystals were filtered, washed with 3 ml. of cold water and air dried, giving 0.038 g., m.p. 122–124°, identical with VII prepared by borohydride reduction of V by mixture melting point determination. Work-up of the solution yielded an additional 3% of the reduction product. Only traces of the quinone V were detected in the remaining solution by paper chromatography. In another experiment, a solution of 0.574 g. of V in 10 ml. of water was allowed to stand in an open flask, exposed to normal laboratory light, for 70 hr. The yield of filtered crystals, m.p. 122–124°, was 0.144 g. (25%), and an additional 2% was recovered from the solution. Other products of the reaction were not investigated.

Identification of products from oxidation of III *to* V. A 0.095*M* solution of III in 40% aqueous acetic acid containing 0.300*N* nitric acid (added as 3*N* fuming nitric acid in acetic acid) at 25° was analyzed periodically. The results are given in Table II.

Nitrous acid was determined colorimetrically.[6] Quinone concentrations were obtained from the absorbance of the diluted solution at 270 mμ (*p*H 6 buffer); correction for the moderate absorption by III at this wave length was made after determining its concentration from its differential absorbance[17] at 290 mμ (*p*H 6 *vs.* *p*H 12 buffers). The *p*H 6 *vs.*

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TABLE II

Time, Min.	Concentration, M	
	Quinone V	HNO_2
0	0	0.001
4	0.020	0.018
10	0.048	0.048
20	0.070	0.072
40	0.074	0.070

*p*H 12 differential absorbance of the quinone at 290 mμ is nearly zero.

One mole of nitrous acid/mole quinone was also produced when the reaction was carried out in chloroform–acetic acid or methylene chloride. Titration of the reaction products in the latter solvent with aqueous sodium hydroxide indicated that V and nitrous acid were the only acidic products. Gas chromatography at room temperature of the reaction mixtures in acetic acid, chloroform–acetic acid, and methylene chloride disclosed the presence of methyl nitrite; although methyl acetate and methanol could have been detected in yields as low as 10%, these compounds were not found. Methyl nitrite was identified as follows: A solution of 0.274 g. of III (0.0015 mole) in 9 ml. of methylene chloride was treated with 1 ml. of 3*N* fuming nitric acid in methylene chloride. After 15 min., a 1-ml. sample of the reaction solution was separated by preparative gas chromatography at room temperature, using the Perkin-Elmer preparative-scale "K" column (polyethylene glycol on firebrick) and nitrogen as the carrier gas. The desired volatile product was collected in a liquid nitrogen trap and then transferred to a 7.5-cm. gas cell with sodium chloride windows. The obtained infrared spectrum was identical with the published[18] spectrum of pure methyl nitrite. After withdrawal of the sample for gas chromatography, the remaining solution of the reaction products was immediately put under vacuum and evaporated at low temperature. One recrystallization of the residue from ether–petroleum ether gave the quinone, m.p. 112–116°, in 66% yield.

In order to determine whether methyl acetate could form from methyl nitrite by transesterification, an oxidation of III in pure acetic acid was carried out with similar reagent concentrations as in the preceding experiment. After a reaction time of 10 min., the quinone V was essentially the only colored compound present in solution (determined by paper chromatography). Gas chromatography again indicated a high yield of methyl nitrite, but no methyl acetate or methanol. To 6 ml. of product solution was added 1 ml. of 70% reagent grade nitric acid. After 1 hr., gas chromatography showed that the concentration of methyl nitrite had decreased more than 50%, while methyl acetate had formed in a yield of at least 30%, based on the initial concentration of III. In a control experiment involving addition of water in place of 70% nitric acid, the concentration of methyl nitrite was reduced less than 10% (after correcting for dilution); methyl acetate was not detected.

The formation of water as a product of the oxidation of III to V in organic solvents was confirmed by gas chromatography on a number of different columns; no quantitative data were obtained, however.

Methyl nitrite from methanol and nitrous acid. A solution of 2.76 g. (0.04 mole) of sodium nitrite in 5 ml. of water was added to 90 ml. of acetic acid containing 0.80 ml. of methanol (0.02 mole), and the volume was made up to 100 ml. by addition of acetic acid. After 10 min., a sample was analyzed by gas chromatography; methyl nitrite was detected in approximately the same concentration as obtained from the oxidation of 0.2*M* solutions of III by 0.4*N* fuming nitric acid in acetic acid.

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Investigations on Lignins and Lignification. XXIV.[1a,b] The Application of Hydrogenation, Hydrogenolysis, and Vapor Phase Chromatography in the Study of Lignin Structure

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Birch and oak milled-wood lignins were subjected to high pressure copper "chromite"-catalyzed hydrogenation and hydrogenolysis under conditions favoring cleavage over reduction. From the hydrogenation reaction mixture, there were isolated and identified by vapor phase chromatography retention times, and infrared spectra: 4-methyl guaiacol, 4-ethyl guaiacol, and 4-*n*-propyl guaiacol, their syringyl analogs, dihydroconiferyl alcohol, and dihydrosinapyl alcohol. Interpretation of these results in terms of C—O and C—C bond hydrogenolysis provides evidence for the presence of arylglycerol β-aryl ether, α-arylhydracrylic aldehyde γ-aryl ether, phenylcoumaran, and β,β'-carbon-carbon type linkages in lignin.

Considerable progress has been made in lignin chemistry since the first hydrogenations[2,3] were carried out on this natural polymer. In addition to increasing our knowledge of its structure,[4] investi-

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