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Kinetics of Iodination. V. Comparison of the Kinetics of Iodination of Isomers and *ortho* Derivatives of 4-Methylphenol*

William E. Mayberry

ABSTRACT: The kinetics of iodination of a series of methylphenol derivatives has been studied as a model for the iodination of tyrosine. The rates of iodination of the position isomers of methylphenol are in the order 3-methylphenol > 2-methylphenol > 4-methylphenol as a result of inductive and presumably steric effects. The second-order rate constant for the iodination of 4-methylphenol is 1.78×10^3 l. mole^{-1} sec^{-1}. On the basis of this rate being 100, the rates for the *ortho*-substituted 4-methylphenols have the following values: 2-nitro, 0.055; 2-bromo, 2.27; 2-iodo, 4.7; 2-allyl, 341; 2-methyl, 345; 2-*n*-propyl, 546; 2-isopropyl, 620. This order is consistent with a primary inductive effect, and the data fit the Hammett $\sigma\rho$ relationship.

The mean value for ρ for the series is -4.664 ± 0.226, reflecting the sensitivity of the iodination reaction to the inductive effect.

In studies comparing the rates of iodination of *N*-acetyl-L-tyrosine and *N*-acetyl-3-iodo-L-tyrosine, the former became iodinated 20–30 times more rapidly than did the latter over the pH range 5.40–9.80. The reason for this was thought to be the inductive effect of the electrophilic iodine atom (Mayberry *et al.*, 1964, 1965a). Similarly, tyrosine has been shown to become iodinated at a rate 17 times greater than that for 3-iodotyrosine (Mayberry *et al.*, 1965b). Berliner *et al.* (1954) have shown that a general inductive effect is responsible for the differences in the relative rates of iodination of a series of *p*-alkylphenols. Both the *p*-alkylphenol studies and the tyrosine studies involved the effect of a substituent on the rates of iodination *meta* to it. The situations were not completely analogous in that the studies with tyrosine involved only an electrophilic substituent (iodine), while Berliner and co-workers' study involved only electron-donating substituents. In addition, the iodine substituent in the tyrosine studies was *ortho* to the hydroxyl group, while in the study by Berliner and co-workers the alkyl groups were *para* to the hydroxyl group.

The present study investigated the effect of a wider variety of *ortho* substituents (R) on the rates of iodination in the other position *ortho* to the hydroxyl group in a series of phenols in which the *para* position was blocked (structure I). Methylphenol derivatives were

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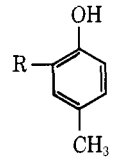

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used because of their availability. The compounds should become iodinated in a manner similar to that for a series of *ortho*-substituted (relative to the hydroxyl group) tyrosines.

Experimental Section

Procedures. KINETIC STUDIES. Runs were performed, in 1-cm quartz cuvets, by following the triiodide concentration spectrophotometrically at 350 mμ as previously described (Mayberry *et al.*, 1964). All experiments, except as noted, were performed with 2×10^{-4} M phenol derivative, 5×10^{-5} M iodine, and 0.12 M iodide. The spectrophotometer chamber holding the cuvets was maintained at 25° by a circulating water bath. Each kinetic run was carried out at a constant volume of 3 ml in 0.045 M sodium borate in 10% methanol (v/v). This concentration of methanol was essential for solubility of the phenolic reactants. The pH of each run was 9.20 as measured (Radiometer TTT1C) with a glass electrode standardized against aqueous buffers. The stability of the triiodide concentration was established by following the optical density for 30 min under the identical conditions of each kinetic run except for the absence of substrate. Each iodination experiment was performed at least in duplicate, and the means and standard deviations for the rates are reported.

The concentration of triiodide could not be followed spectrophotometrically during iodination of 2-nitro-4-methylphenol because this compound absorbs light strongly at 350 mμ. In these experiments, the iodine concentration was followed by thiosulfate titration (thiosulfate solutions were standardized against KIO_3 and found to be 0.0985 N). To 75 ml of 2.4×10^{-4} M 2-nitro-4-methylphenol in borate buffer in a water-jacketed reaction vessel maintained at 25° was added 15 ml of 3.0×10^{-4} M I_2 in 0.02 M iodide in borate buffer at 25°; the system was mixed by magnetic stirring. Time was measured from the first addition of the iodine solution. Aliquots of the reaction mixture were removed at intervals and added to 1 N HCl (to stop the reaction) in an erlenmeyer flask; 1 ml of 1% soluble starch in 2% KI was added, a stopper was placed on the flask, and a small polyethylene tube was introduced through a hole in the stopper. The solution was then titrated with an appropriate dilution of the standard thiosulfate solution. Duplicate runs were made in each instance.

RATE CONSTANT DETERMINATIONS. Rate constants for each run were determined from 20 to 40 optical density readings according to the integrated second-order equation derived (Mayberry *et al.*, 1964) for computer (IBM 7094) solution

$$OD = \alpha\left[b - \frac{e^{k_{obsd}(a-b)t} - 1}{e^{k_{obsd}(a-b)t} - b/a} \times b\right] \quad (1)$$

in which t = time in seconds, a and b are the stoichiometric concentrations of phenol derivative and iodine (at time = 0), and α is a proportionality constant which converts optical density (OD) into concentration of iodine at time t. Observed rate constants for the iodination of 2-nitro-4-methylphenol were calculated by the standard graphic method using the integrated second-order equation.

The observed rate constants have been corrected for variation in iodide and phenolate concentrations according to

$$k = \frac{k_{obsd}(K_1 + [I^-])(K_2 + [H^+])[I^-]}{K_1K_2} \quad (2)$$

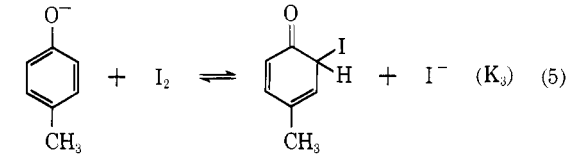
in which K_1 and K_2 are equilibrium dissociation constants for the reactions

$$I_3^- \rightleftharpoons I_2 + I^- \text{ for } K_1^1 \quad (3)$$

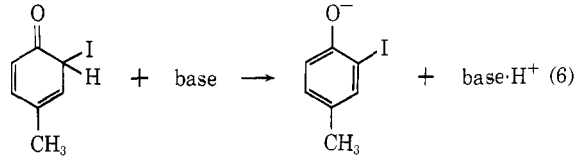
and

$$PhOH \rightleftharpoons PhO^- + H^+ \text{ for } K_2 \quad (4)$$

The two $[I^-]$ terms in the numerator of eq 2 are necessary for correction of observed rate constants because of the preequilibria depicted in eq 3 and 5.



(5)



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The second iodide correction term requires that K_3 be quite small with respect to the iodide concentration in the system; this seems quite likely to be true (Mayberry *et al.*, 1964).

DETERMINATION OF APPARENT HYDROXYL pK. The apparent pK value for the hydroxyl group of each methylphenol compound was determined by spectrophotometric titration. Determinations were carried out in 0.045 M sodium borate buffer in 10% methanol in 0.12 M NaCl. Titration was performed, in cuvets, with both 2 N HCl and 1 N NaOH in 10% methanol, delivered with an Agla micrometer syringe through

[1] K_1 = 0.0013 at 25° (Davies and Gwynne, 1952).

a small-bore polyethylene tube and mixed with magnetic stirring. pH determinations were made with "miniature" glass electrodes.[2] The optical density of the methylphenols and *ortho*-substituted 4-methylphenols was followed at 293 mμ, the λ_{max} of the compounds in alkali. The halogenated 4-methylphenols were followed at 305 mμ, λ_{max} in alkali; the nitro compound, λ_{max} 436 mμ, was followed at 430 mμ.

SPECTRA. Ultraviolet spectra (Cary Model 15) were determined for some compounds in 0.1 N HCl and in 0.1 N NaOH, both in 10% methanol. Spectra for all of the phenol compounds were obtained in borate buffer in 10% methanol. The infrared spectrum[3] of each compound was determined in cyclohexane with sodium chloride optics. Nuclear magnetic resonance (nmr) spectra[3] of the compounds were determined in deuterated trichloromethane as solvent with tetramethylsilane as an internal standard.

GAS CHROMATOGRAPHY. The homogeneity of each compound was determined by gas chromatography (Barber-Colman Model 10) with acetone as the solvent. A 4-m column of Gas Chrom P, 60–80 mesh and coated with 30% SE-30 polymer, was used. The trifluoroacetate derivative of each phenolic compound also was prepared and chromatographed.

Materials. 4-METHYLPHENOL. Redistilled 4-methylphenol (Matheson Co.) was dissolved in petroleum ether (bp 37–53°). Crystallization was initiated by immersing the flask containing the solution into a Dry Ice–acetone bath and then was allowed to proceed at 10°. The white crystals were filtered on sintered glass at 10°, recrystallized, washed with cold petroleum ether, and dried in the cold in a vacuum desiccator for 24 hr (mp 32.5–33.5°;[4] lit. (Knapp *et al.*, 1950) mp 34.3°).

3-METHYLPHENOL. Redistilled 3-methylphenol (Matheson Co.), a rust-colored liquid as purchased, was dissolved in petroleum ether; crystallization was initiated as above and then was completed at −10°. The resulting white crystals were filtered, washed, recrystallized four times, and dried in the cold in a vacuum desiccator for 24 hr (capillary tube mp 12.5–13.5°; lit. (Knapp *et al.*, 1950) mp 11.3°).

2-METHYLPHENOL. This compound (Fisher Scientific Co.) was used as purchased without further purification.

2-NITRO-4-METHYLPHENOL. This compound (Aldrich Chemical Co.) was purchased as yellow-brown crystals which were dissolved in toluene and recrystallized at −40°. The yellow crystals were washed with toluene at −10° and dried in a vacuum desiccator (mp 33.5–34.5°). After further recrystallization from ethyl ether at −10° there was no change in melting point (lit. (Brasch and Freyss, 1891) mp 36.5°). *Anal.* Calcd for $C_7H_7NO_3$: C, 54.90; H, 4.60; N, 9.15. Found: C, 54.84; H, 4.55; N, 9.09.

2-BROMO-4-METHYLPHENOL. The compound (Aldrich Chemical Co.) was purchased as a yellow liquid. Silky, white needles were recrystallized three times and dried by the procedures used for 4-methylphenol (capillary tube mp 19.5–20°, lit. (Vogt and Henninger, 1882) mp 17–18°). *Anal.* Calcd for C_7H_7BrO: C, 44.95; H, 3.77; Br, 42.72. Found: C, 44.69; H, 3.91; Br, 43.02.

2,4-DIMETHYLPHENOL. This compound (Aldrich Chemical Co.), purchased as a clear oil, was twice recrystallized and dried as for 4-methylphenol and yielded white needles (capillary tube mp 25.5–26°; lit. (Jacobsen, 1885; Skita, 1922) mp 26°).

2-ISOPROPYL-4-METHYLPHENOL. The compound (Aldrich Chemical Co.) was purchased and redistilled under vacuum. The yellow fraction, bp 78° (1 mm), was collected (lit. (Carpenter and Easter, 1955) bp 82 (3 mm) and (Guillaumin, 1910) 228–229° (763 mm)).

2-*n*-PROPYL-4-METHYLPHENOL. The clear liquid (Aldrich Chemical Co.) was purchased and vacuum distilled. A clear fraction, bp 70° (1 mm), was collected (lit. (Braun *et al.*, 1924) bp 97° (13 mm)).

2-ALLYL-4-METHYLPHENOL. The clear oil (Aldrich Chemical Co.) was used as purchased without further purification. *Anal.* Calcd for $C_{10}H_{12}O$: C, 81.04; H, 8.16. Found: C, 81.08; H, 8.33.

2-AMINO-4-METHYLPHENOL. The recrystallized 2-nitro-4-methylphenol (4 g) was dissolved in 100 ml of 5% aqueous sodium hydroxide (producing a bright red solution) and was heated to boiling. Sodium hydrosulfite was added in small portions with stirring until the color disappeared (about 15 g). On cooling, a white, feathery precipitate formed. The reaction mixture was overlaid with nitrogen and extracted three times with peroxide-free ether. The combined ether extracts were washed with water and dried over sodium sulfate. On evaporation of the ether, 3 g (94%) of white, feathery crystals was obtained (mp 135–135.5°; lit. (Nölting and Kohn, 1884) mp 135°).

2-IODO-4-METHYLPHENOL. A solution of 1.9 g (27.5 mmoles) of sodium nitrite in 10 ml of water was added dropwise at −5° to a vigorously stirred mixture of 3 g (24 mmoles) of 2-amino-4-methylphenol and 32 ml of 20% (v/v) aqueous sulfuric acid. Stirring was continued to 20 min in an ice bath. The reaction mixture was rinsed, with 20 ml of 20% (v/v) aqueous sulfuric acid, into 5 ml of aqueous solution containing 5 g (30 mmoles) of potassium iodide. The resulting solution was vigorously stirred and heated for 10 min in a water bath at 50° and then for 40 min at 85–90°. (A cold-finger condenser was used.) Evolution of nitrogen began when the bath temperature reached about 85° and ceased 30 min thereafter. Stirring was continued until the reaction mixture had cooled to room temperature (90 min). The condensate on the cold finger was rinsed into the reaction flask with ether, and the iodine formed was reduced with sodium metabisulfite.

[2] Leeds & Northrup electrodes adapted for use with the Radiometer TTT1C.

[3] Infrared and nmr spectra were obtained with the Beckman IR-7 and Varian A-60, respectively. Details of the spectral identification for the compounds may be obtained by writing to the author.

[4] Melting points were taken on a Kofler stage unless otherwise indicated. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

The mixture was extracted with ether, and the ether extract was dried over sodium sulfate and evaporated. The dark, oily residue was steam distilled and the distillate was collected in several fractions. The first fraction, containing the bulk of the iodophenol, was extracted with ether. The ether fraction was dried and evaporated. On cooling a solution of the residues in petroleum ether (bp 39–52°) in a Dry Ice–acetone bath, crystals began to form. Crystallization was then allowed to continue at 4°. The crystals were collected by filtration, washed with cold petroleum ether, and dried; yield 1 g, 21%; mp 35–36°; lit. (Dimroth, 1902) mp 35° and (Morgan and Burstall, 1928) 37–38°. *Anal.* Calcd for C_7H_7IO: C, 35.92; H, 3.01; I, 54.22. Found: C, 35.76; H, 2.71; I, 54.85.

Recrystallization from petroleum ether did not raise the melting point. A second crop was obtained from the later fractions of the steam distillation.

TABLE I: Gas Chromatographic Retention Times of Isomers and *ortho*-Substituted Derivatives of 4-Methylphenol.

Compound	Retention Time (min)	Conditions[a]
4-Methylphenol	10.98	1
4-Methylphenyl trifluoroacetate	15.18	2
3-Methylphenol	10.98	1
3-Methylphenyl trifluoroacetate	14.46	2
2-Methylphenol	9.92	1
2-Methylphenyl trifluoroacetate	12.11	2
2-Nitro-4-methylphenol	10.63	3
2-Nitro-4-methylphenyl trifluoroacetate	12.05	3
2-Bromo-4-methylphenol	8.74	3
2-Bromo-4-methylphenyl trifluoroacetate	8.03	3
2-Methyl-4-methylphenol	6.97	3
2-Methyl-4-methylphenyl trifluoroacetate	4.72	3
2-*n*-Propyl-4-methylphenol	11.93	3
2-*n*-Propyl-4-methylphenyl trifluoroacetate	7.68	3
2-Isopropyl-4-methylphenol	10.87	3
2-Isopropyl-4-methylphenyl trifluoroacetate	6.97	3
2-Iodo-4-methylphenol	11.70	3
2-Iodo-4-methylphenyl trifluoroacetate	10.65	3

[a] Argon flow at 16 psi. 1 = cell, 255°; column, 137°; flash heater, 187°. 2 = cell, 245°; column, 110°; flash heater, 177°. 3 = cell, 255°; column, 179°; flash heater, 245°.

Results

Chromatographic Analysis of Reactants. The analytic and spectral data obtained for each phenolic compound suggested that the compounds were free of impurities. The data further offered some proof that position isomers, if present, were not in sufficient concentration to be detected. To test the latter possibility further, each compound was analyzed by gas chromatography. The retention time for each phenol derivative is presented in Table I. Chromatographic conditions were found that allowed separation of 4-methylphenol and 3-methylphenol from 2-methylphenol. Separation of 4-methylphenol from 3-methylphenol was not possible with a wide range of conditions; however, the trifluoroacetate derivatives of these could be separated. Each of the *ortho*-substituted 4-methylphenols and each of their trifluoroacetate derivatives gave a sharp, single peak. None of the position isomers of the *ortho*-substituted compounds was available for study. Nevertheless, if we assume that their behavior on gas chromatography is similar to that of the isomers of methylphenol, then the present data offer further proof that position isomers were not present to any significant degree.

The trifluoroacetate derivative of each *ortho*-substituted 4-methylphenol had a shorter retention time than did the parent compound, with a single exception, 2-nitro-4-methylphenyl trifluoroacetate. The spectral data showed that 2-nitro-4-methylphenol has a strong intramolecular hydrogen bond, and conversion to the phenyl trifluoroacetate may thus allow more ionic interaction of the compound with the polymer and support in the column and, consequently, retard flow.

Apparent Hydroxyl pK. The apparent hydroxyl pK for each phenolic compound was determined because the calculated rate constants are quite sensitive to the degree of ionization. These data are listed in Table II. The values found for the isomers of methylphenol are similar to those reported by Boyd (1915):

TABLE II: Apparent Hydroxyl pK of Isomers and *ortho*-Substituted Derivatives of 4-Methylphenol.[a]

Compound	pK_a
4-Methylphenol	10.17
3-Methylphenol	10.06
2-Methylphenol	10.32
2,4-Dimethylphenol	10.51
2-*n*-Propyl-4-methylphenol	10.72
2-Isopropyl-4-methylphenol	10.73
2-Allyl-4-methylphenol	10.47
2-Iodo-4-methylphenol	8.88
2-Bromo-4-methylphenol	8.77
2-Nitro-4-methylphenol	7.41

[a] Determined by spectrophotometric titration in $Na_2B_4O_7$ buffer, pH 9.20, with 10% methanol (v/v).

10.17, 10.01, 10.20, and 10.47 for 4-methylphenol, 3-methylphenol, 2-methylphenol, and 2,4-dimethylphenol, respectively. The value for 2-methylphenol is close to that reported by Sprengling and Lewis (1953). The values for the other *ortho*-substituted derivatives in general seem to be internally consistent. The alkyl substituents decrease the acidity and the halide and nitro groups increase the acidity of the phenols. The 2-*n*-propyl substituent has a greater effect than the 2-methyl substituent, as would be expected on the basis of the greater electron-donating capacity of the propyl group. The value for the 2-isopropyl derivative is about the same as for the *n*-propyl. The value for the 2-allyl compound is less than that for the propyl compounds, compatible with its decreased electron-donating characteristics resulting from its unsaturation.

Fit of Data to Second-Order Equation. An example of the fit of the observed data to the second-order rate equation (eq 1) is shown in Table III for the iodination of 4-methylphenol. The fit of the data for the other iodination reactions was equally good. In the example shown, the observed velocity constant is 15.58 l. mole^{-1} sec^{-1}. In this situation, α (24.79×10^3) approaches the extinction for triiodide ion at the iodide concentration of 0.12 M and stoichiometric iodine concentration of 5×10^{-5} M. The extinction, ϵ, for triiodide is reported as 26.4×10^3 at 352 mμ (Allen and Keefer, 1955). The identification of products of phenolic iodination has been investigated previously (Mayberry *et al.*, 1964) and will be discussed subsequently with respect to the present data.

Rate Constants for Iodination. The rate constants for iodination of the position isomers and the *ortho* derivatives of 4-methylphenol are listed in Table IV.

TABLE III: Computer Print-Out for Iodination of 4-Methylphenol at 25°.

Time (sec)	Y × 10^4 [a]	Optical Density: Calcd	Obsd	Calcd/Obsd
25	0.4629	1.145	1.150	0.998
39	0.4436	1.100	1.100	1.000
55	0.4227	1.048	1.050	0.998
71	0.4031	0.999	1.000	0.999
84	0.3879	0.962	0.960	1.002
97	0.3734	0.926	0.920	1.006
112	0.3575	0.886	0.880	1.007
122	0.3473	0.861	0.860	1.001
132	0.3375	0.837	0.840	0.996
140	0.3298	0.818	0.820	0.997
157	0.3143	0.779	0.780	0.999
166	0.3064	0.760	0.760	1.000
176	0.2979	0.739	0.740	0.998
185	0.2905	0.720	0.720	1.000
195	0.2826	0.701	0.700	1.001
206	0.2741	0.680	0.680	1.000
216	0.2666	0.661	0.660	1.002
228	0.2580	0.640	0.640	1.000
240	0.2497	0.619	0.620	0.998
253	0.2410	0.597	0.600	0.996
265	0.2333	0.578	0.580	0.997
278	0.2253	0.558	0.560	0.997
291	0.2175	0.539	0.540	0.999
304	0.2101	0.521	0.520	1.002
318	0.2024	0.502	0.500	1.004
334	0.1940	0.481	0.480	1.002
342	0.1900	0.471	0.470	1.002

α = 24,793; k_{obsd} = 15.58 ± 0.04

[a] Y is equal to the bracketed term in eq 1.

TABLE IV: Rate Constants for Iodination of Isomers and *ortho*-Substituted Derivatives of 4-Methylphenol at 25°.

Compound	k_{obsd} ± Std Dev[a] (l. mole^{-1} sec^{-1})	k	Rel Rates
4-Methylphenol	15.58 ± 0.04	1,781	100
2-Methylphenol	30.33 ± 0.29	4,783	269
3-Methylphenol	104.74 ± 0.48	9,667	543
2,4-Dimethylphenol	25.63 ± 0.06	6,148	345
2-*n*-Propyl-4-methylphenol	25.46 ± 0.08	9,729	546
2-Isopropyl-4-methylphenol	28.28 ± 0.23	11,047	620
2-Allyl-4-methylphenol	28.10 ± 0.12	6,076	341
2-Iodo-4-methylphenol	5.02 ± 0.09	8,312	4.7
2-Bromo-4-methylphenol	2.64 ± 0.08	40.47	2.27
2-Nitro-4-methylphenol	2.99[b]	0.99	0.055

[a] Conditions were: 4-methylphenol or derivative, 2×10^{-4} M; I_2, 5×10^{-5} M; I^-, 0.12 M; $Na_2B_4O_7$, 0.045 M; pH 9.20; methanol, 10%. [b] I^- was 0.02 M in these runs. I_2 concentration was measured by thiosulfate titration.

The corrected rate constant for the iodination of 4-methylphenol is 1781 l. mole^{-1} sec^{-1}; the relative rates for the other compounds were calculated on the basis of this value being 100 and are given in column 3 of Table IV. The values for the position isomers, 2-methylphenol and 3-methylphenol, are 2.7 and 5.4 times greater than the value for the 4-methylphenol compound. Furthermore, all of the *o*-alkyl-substituted 4-methylphenol compounds had an iodination rate constant three to six times greater than that of the

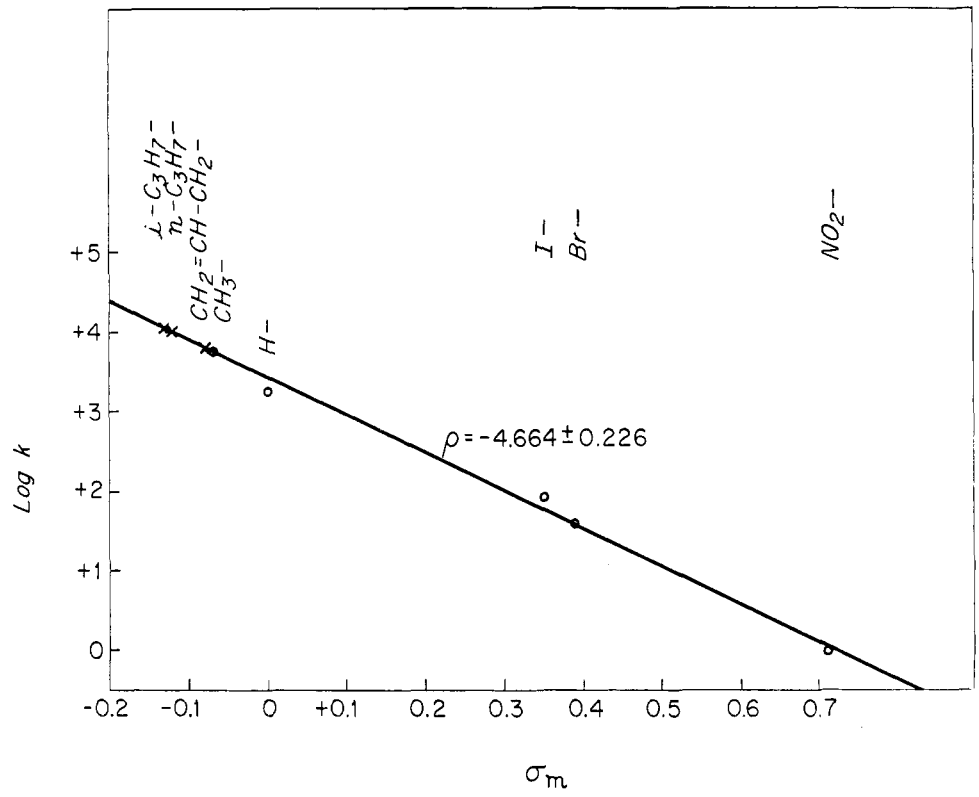

FIGURE 1: Hammett plot of log k for iodination of *ortho*-substituted 4-methylphenols against *meta* σ constant for that substituent. (circles) Points with known σ constants; (x) σ constants calculated from eq 7.

parent compound. Conversely, the *o*-halogen and *o*-nitro compounds had rate constants $^1/_{20}$ to $^1/_{1800}$ of that of 4-methylphenol. As expected, these values almost surely reflect the electron-donating effects of the alkyl substituents and the electron-withdrawing effects of the halogen and nitro substituents.

Linear-Free-Energy Relationship. Because the rate constants in Table IV for the iodination of 4-methylphenol and of *ortho*-substituted compounds seemed to reflect inductive effects, the correlation of the data with the Hammett $\sigma\rho$ relationship (Hammett, 1935) (eq 7) was tested.

$$\log k - \log k^\circ = \sigma\rho \qquad (7)$$

A plot of the log k for 4-methylphenol and various substituents of 4-methylphenol is shown in Figure 1. *meta* σ constants were not available for the substituents isopropyl, *n*-propyl, and allyl. A plot of the other values (Hammett, 1940) defined a reasonably straight line. The line drawn represents the least-squares fit of the data for the five known *meta* σ constants. The slope of this line, ρ, is -4.664 ± 0.226. Because ρ is a characteristic of the reaction series, the values for the unknown *meta* σ constants may be calculated from eq 7: -0.133, -0.123, and -0.079 for isopropyl, *n*-propyl, and allyl, respectively. On the basis of the present data and the other Hammett σ constants, these latter values seem theoretically reasonable.

Effect of Heat on Reaction Rates. Except for 2-methylphenol, the rate of each iodination reaction was determined at 20, 27, and 30° in addition to 25°. In the calculation of k at 20, 25, and 30°, known appropriate values for K_1 were used (Katzin and Gebert, 1955). K_1 at 27° was estimated by interpolation. Since apparent values for K_3 are known only at 25°, these values were used at all of the temperatures.

The Arrhenius activation energies for the reactions are given in Table V. These values are not true activation energies but composite terms which include the ΔH^* for ionization of 4-methylphenol or its derivative and for the buffer constituents. The value for 4-methylphenol was 20.3 ± 0.3 kcal mole^{-1} and for 3-methylphenol about 3 kcal mole^{-1} less. The values of E_a for iodination of the 2-methyl, 2-isopropyl, and 2-allyl derivatives were 2–3 kcal mole^{-1} less than that for 4-methylphenol, while the values for the remaining compounds were approximately the same as for 4-methylphenol.

ΔS^* has been calculated as described by Bunnett (1961) and the values are listed in Table V. All of the values are positive, reflecting the fast rates of these reactions. Whether there is significant difference among the values of the series is uncertain; the value for 2-nitro-4-methylphenol is probably significantly less than the other values.

TABLE V: Activation Energies for Iodination of Methylphenols and Derivatives at pH 9.20.

Compound	E_a[a] (kcal mole^{-1})	ΔS^*[b] (eu)
4-Methylphenol	20.3 ± 0.3	+22.4
3-Methylphenol	16.9 ± 0.7	+19.1
2,4-Dimethylphenol	18.1 ± 0.5	+17.6
2-*n*-Propyl-4-methylphenol	19.8 ± 0.5	+24.3
2-Isopropyl-4-methylphenol	17.7 ± 0.4	+17.4
2-Allyl-4-methylphenol	17.5 ± 0.2	+15.6
2-Iodo-4-methylphenol	20.4 ± 0.5	+16.7
2-Bromo-4-methylphenol	21.1 ± 0.6	+17.6
2-Nitro-4-methylphenol	19.4[c]	+4.7

[a] The plus and minus standard deviation was determined by the method of least squares. [b] At 25°. [c] Calculated from values at only two temperatures.

Discussion

Evidence has been presented previously (Mayberry *et al.*, 1964) to show that the phenol derivative, *N*-acetyl-L-tyrosine, is diiodinated to only a very slight extent when the stoichiometric ratio of I_2 to phenol derivative is about 1. In the present experiments, the phenolic reactants in a stoichiometric concentration four times that of I_2 did not become diiodinated to any appreciable extent, probably as a result of both steric and inductive effects. Therefore, the fact that 2-methylphenol is iodinated 2.7 times faster than 4-methylphenol reflects the greater facility for *para* iodination on the phenolate anion than for *ortho* iodination. It is known that, in general, *para* halogenation is favored over *ortho* halogenation (Holleman, 1924). For chlorination of phenol, the *ortho*:*para* ratio is 1.0 although there are two *ortho* sites but only one *para* site on the phenol molecule. For bromination, the ratio is 0.1. Thus, it seems reasonable that, as the size of a substituent becomes larger, *para* substitution will be favored (Alexander, 1950). Because the iodine substituent is larger than either bromine or chlorine, the present assumption that iodination of 2-methylphenol is occurring primarily, if not entirely, in the *para* position is very reasonable.

The fact that 3-methylphenol iodinates at a rate 5.4 times that of 4-methylphenol also offers evidence for the facility of iodination at the *para* position compared to the *ortho* position. In addition, since the rate for 3-methylphenol is twice that of 2-methylphenol, the inductive effect and, perhaps, the resonance effect, of the methyl group adjacent to the position *para* to the hydroxyl group must further facilitate the iodination reaction. It is well known that inductive effects suffer loss in relay (Ingold, 1953).

The fit of the data for *ortho*-substituted 4-methylphenols to the Hammett relationship is good. The rates of iodination of phenolic compounds are thus influenced primarily by the inductive effect, as assumed previously (Mayberry *et al.*, 1964). The negative value for ρ in the present series indicates that the reaction rates in the series are decelerated by electron-withdrawing substituents and accelerated by electron-donating substituents. The magnitude of ρ indicates the sensitivity to the electronic effects. In the present instance, the value of -4.66 ± 0.22 is considerably more negative than for any reaction series reviewed initially by Hammett (1940). However, the value is similar in magnitude to the values compiled by Brown and Okamoto (1958) for the ρ constants of electrophilic aromatic substitution reactions.

Since there is generally conceded to be an inherent error of 1–2 kcal mole^{-1} in reaction rate energy terms, the activation energies for iodination in the present series of compounds may be considered to be roughly equivalent. It may well be that significant differences in the individual values are hidden by the methods, because E_a reflects the ΔH^* for the phenolic reactant and the sum of two reactions represented by eq 5 and 6. However, there is a significant difference between the value for ΔS^* for 2-nitro-4-methylphenol and that for any of the other compounds. Since the entropy of activation reflects in part a probability factor that molecules having the necessary energy will react upon collision, it may be assumed that, for 2-nitro-4-methylphenol, significantly fewer molecules do react than for the other compounds.

The findings in the present study are of some biologic importance because the reactions with methylphenols may serve as chemical models for the iodination of tyrosine within thyroglobulin. In this context, it is also of interest that the ratio of rate of iodination of 4-methylphenol to that of 2-iodo-4-methylphenol is 21, a value similar to the ratio between tyrosine and monoiodotyrosine (Mayberry *et al.*, 1965b).

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Completely Deuterated Proteins. III. Deuteration Effects on Protein–Protein Interaction in Phycocyanin*

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ABSTRACT: The state of aggregation of fully deuterated phycocyanin is studied as a function of pH, temperature, and ionic strength by sedimentation velocity measurements. Studies of the pH dependence of viscosity and absorption spectra are also presented. The data are compared to those for the normal phycocyanin (Scott, E., and Berns, D. S., *Biochemistry 4*, 2597 (1965)). Under comparable conditions the deuterio protein contains less 11S material and very little 19S material and an enhanced amount of 7S and 3S species. Possible explanations for the results include conventional isotope effects, hydrophobic bonding, and van der Waal's interaction. It is suggested that the 11S aggregate is normally stabilized by dispersion forces in combination with hydrophobic and other forces competing with electrostatic repulsion between the identical subunits, and that in the deuterated protein there is a decrease in polarizability of the groups involved in the protein–protein interaction. More efficient competition of the electrostatic repulsion results in a decreased stability of 11S species with the appearance of more 7S material.

In previous papers in this series (Berns, 1963a,b), we have demonstrated that C-phycocyanins extracted and purified from the blue-green alga *Plectonema calothricoides*, grown in H_2O and in 99.8% D_2O, were antigenically identical but differed in their thermal denaturation characteristics. The aggregation properties of C-phycocyanin have been recently studied in detail, as a function of pH, ionic strength, and temperature (Scott and Berns, 1965), and we now have completed an analogous study on the fully deuterated protein in H_2O. Hattori *et al.* (1965) have recently published a report on the aggregation in the same system and the effects of deuterium substitution. The two studies will be compared where feasible.

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Experimental Section

Materials and Methods. In most of our studies fully deuterated phycocyanin from *P. calothricoides* was used; however, phycocyanin from *Phormidium luridum* was also employed. Previous work (Scott and Berns,