[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, CASE INSTITUTE OF TECHNOLOGY]

The Reduction of Substituted Nitrobenzenes at the Dropping Mercury Cathode

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Substituted nitrobenzenes were among the first organic compounds to be investigated using the polarographic technique. The early work was performed by the Japanese, Shikata and his co-workers, and from this work Shikata and Tachi[3] formulated the "electronegativity rule of reduction potentials," *i. e.*, that organic compounds are more easily reduced as more electronegative groups are substituted in the same molecule.

These investigators measured the reduction potentials by the tangent method *vs.* the normal calomel electrode. This method of measurement deviates from present practice. It was felt desirable to extend their investigations of substituted nitrobenzenes and to determine the relative effect of the position of the substituent upon the reduction process.

The ortho-, meta- and para-isomers of nitrobenzoic acid, dinitrobenzene, nitrochlorobenzene, and nitrotoluene were selected for study. The reduction of these compounds at the dropping mercury cathode from carefully buffered solutions over the *p*H range 2 to 14 was investigated to determine the nature of the reduction process and the effect of the position of the substituent groups upon the half-wave reduction potential.

Experimental

All of the compounds investigated were obtained from Eastman Kodak Company and were further purified before use. Water solutions of 0.002 *M* concentration of *o*- and *m*-nitrobenzoic acids and of 0.001 *M* concentration of *p*-nitrobenzoic acid were made up for use as master solutions. The dinitro-, chloronitro- and nitrotoluenes were made up as 0.002 *M* solutions in alcohol. Gelatin was incorporated into each of the master solutions as a water solution of such concentration that the sample for electrolysis (diluted 1:1 with buffer) would contain 0.0075% gelatin.

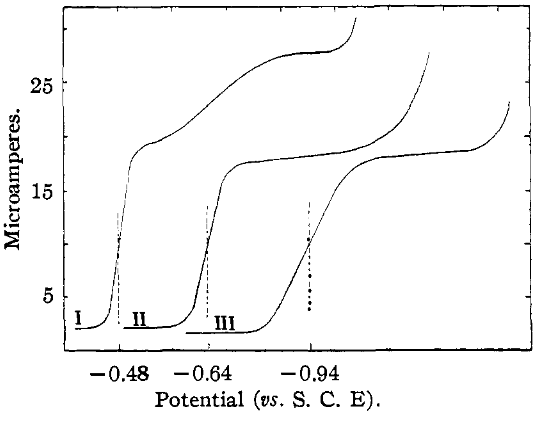

Fig. 1.—Polarograms for *o*-nitrobenzoic acid: I, *p*H 2.1; II, *p*H 4.0; III, *p*H 6.8.

For *p*H values up to 8 McIlvaine's citric acid–disodium phosphate buffers were used as supporting electrolyte and for *p*H values greater than 8, Sörensen's glycine–sodium hydroxide buffers were used. The *p*H of each solution was checked with a Beckman *p*H meter.

The sample to be electrolyzed was prepared by mixing equal parts of the master solution of nitro-compound and the desired buffer solution. This solution was degassed in the electrolysis cell by passing nitrogen through the solution for seven to eight minutes. The electrolysis was carried out in a constant temperature bath at 25°.

An H-type cell containing a saturated calomel working electrode was used in conjunction with a Sargent Model XX Polarograph. The drop time of the capillary was found to be 3.2 seconds per drop and the value of $m^{2/3}t^{1/6}$ was 2.13 mg.$^{2/3}$sec.$^{-1/2}$ measured in water with no applied potential.

Discussion of Results

The wave heights and half-wave potentials determined for the nitrobenzoic acids are tabulated in Table I. Inspection of the table reveals that all of the wave heights are approximately 15 microamperes for a 0.001 molar concentration of the acid. Using the value 8.28×10^{-6} cm.^{2} sec.$^{-1}$ for the diffusion coefficient of nitrobenzoic acid the value of *n* in the Ilkovic equation is found to be 4.05 which indicates that the reduction of the nitro group proceeds only to the hydroxylamine. The diffusion coefficient was determined from the equivalent conductance of the nitrobenzoate ion at infinite dilution. Representative polarograms obtained with *o*-nitrobenzoic acid are shown in Fig. 1. The other two isomers gave similar curves.

TABLE I

HALF-WAVE REDUCTION POTENTIALS (VOLTS *versus* THE S. C. E. CORRECTED FOR *IR* DROP) AND DIFFUSION CURRENTS (MICROAMPERES) FOR AQUEOUS SOLUTIONS OF NITROBENZOIC ACIDS

	Ortho[a]		Meta[b]		Para[a]	
*p*H	*E*	*I*	*E*	*I*	*E*	*I*
2.1	−0.48	15.2	−0.45	15.0	−0.41	7.1
4.0	−0.64	15.5			−0.51	8.0
5.05			−0.64	15.5		
6.0	−0.85	15.3			−0.63	7.8
8.1	−1.06	15.4	−0.83	15.0		
10.0	−1.26	15.2	−0.97 −1.51	7.7 7.7 [c]	−0.84	7.9
12.2	−1.36	15.8	−1.01 −1.42	7.7 7.7	−0.95	7.9

[a] 0.001 *M* solutions. [b] 0.0005 *M* solutions. [c] Waves bracketed together were double waves.

Data for the dinitrobenzenes are presented in Table II. The presence of two reducible groups makes the interpretation of the reaction mechanism somewhat obscure, since multiple waves are encountered. Some of the waves following the

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(1) From the M. S. thesis of Sally Free Dennis.

(2) Present address: Lubrizol Corp., Wickliffe, Ohio.

(3) M. Shikata and I. Tachi, *J. Chem. Soc. Japan*, **53**, 834 (1932); *Coll. Czech. Chem. Commun.*, **10**, 368 (1938).

TABLE II

HALF-WAVE REDUCTION POTENTIALS (VOLTS *versus* THE S. C. E. CORRECTED FOR *IR* DROP) AND DIFFUSION CURRENTS (MICROAMPERES) FOR 0.001 *M* 50/50 ETHYL ALCOHOL–WATER SOLUTIONS OF DINITROBENZENES

	Ortho		Meta		Para	
*p*H	*E*	*I*	*E*	*I*	*E*	*I*
2.7	−0.43	11.1	−0.45	12.4	−0.39	9.4
	−0.65	20.8	−0.60	12.4	−0.70	21.2
	−1.28	5.2	−1.13	7.2		
4.6	−0.54	14.0	−0.55	13.0	−0.48	11.4
	−0.81	13.7	−0.78	10.8	−0.89	19.5
	−1.48	9.5	−1.64	11.0		
7.4	−0.66	12.2	−0.71	14.0	−0.60	13.0
	−1.00	15.4	−0.96	11.4	−1.08	19.5
8.8					−0.63	12.6
					−1.15	10.9
					−1.73	16.6
10.0	−0.73	12.0	−0.86	11.7	−0.65	12.0
	−1.16	20.8	−1.10	16.9	−1.21	17.6
					−1.80	8.8
11.7	−0.76	11.4	−0.88	12.7	−0.65	6.2
	−1.29	16.6	−1.16	11.7	−1.76	24.4

first are not sufficiently well defined to make their interpretation possible with any degree of accuracy (Fig. 2), however, if correction is made for the effect on wave height of the alcohol required to increase solubility, the first wave is found to be approximately the height of that observed for the nitrobenzoic acid and therefore must represent reduction to the corresponding hydroxylamine. The alcohol effect was measured by reducing nitrobenzoic acid both from water and from 50% alcohol solutions. The ratio between wave heights for the same compound in the two solutions was about 5:4 which corresponds fairly well to the ratio of the nitrobenzoic acid to dinitrobenzene (first wave) heights.

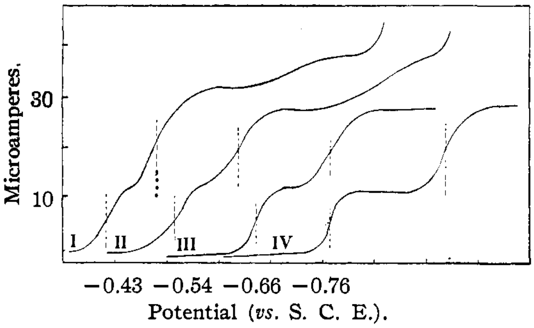

Fig. 2.—Polarograms for *o*-dinitrobenzene: I, *p*H 2.7; II, *p*H 4.6; III, *p*H 7.4; IV, *p*H 11.7. The divisions represent 0.25-volt intervals.

The half-wave potentials for the first and second waves plotted against *p*H are shown in Fig. 3. There is some doubt as to the exact potential of the second wave for the meta-isomer because of difficulty in measurement. It is noted that the order of ease of reduction is para > ortho > meta for the first wave and exactly the reverse for the second wave. This seems reasonable when one considers that the second reduction step takes place under the influence of the hydroxylamine group which is less electronegative than the nitro group. Also the hydroxylamine group is capable of hydrogen bonding with the ortho-nitro group and this would lead to increased ease of reduction of the ortho-compound as has been previously observed in the case of the nitrophenols.[4]

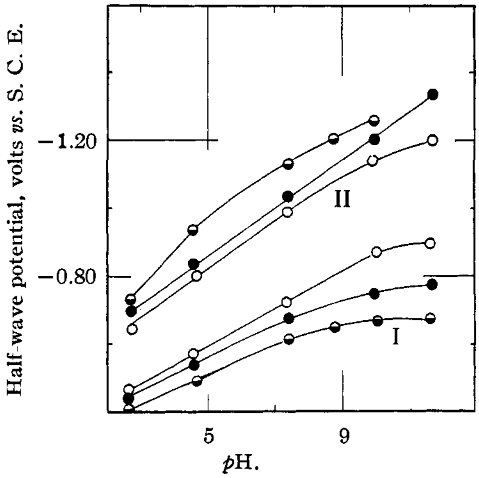

Fig. 3.—Effect of *p*H on the half-wave reduction potentials of the dinitrobenzenes: first wave I, and second wave II: ⊖, para; ●, ortho; ○, meta.

As with the nitrobenzoic acids, the data indicate that the nitrotoluenes are reduced to the hydroxylamines at all *p*H values (Table III). In the acid solutions the ortho isomer is considerably more difficultly reduced than the meta- and para-isomers, and the order of ease of reduction changes in alkaline solution to meta > ortho > para. The current–voltage curves were similar to those shown for *o*-nitrobenzoic acid.

TABLE III

HALF-WAVE REDUCTION POTENTIALS (VOLTS *versus* THE S. C. E. CORRECTED FOR *IR* DROP) AND THE DIFFUSION CURRENTS (MICROAMPERES) FOR 0.001 *M* 50/50 ETHYL ALCOHOL–WATER SOLUTIONS OF NITROTOLUENES

	Ortho		Meta		Para	
*p*H	*E*	*I*	*E*	*I*	*E*	*I*
2.7	−0.69	11.0	−0.65	11.9	−0.63	11.4
4.6	−0.84	11.4	−0.74	12.1		
5.65					−0.83	12.4
7.4	−0.99	11.3	−0.93	12.1	−0.95	12.4
10.0	−1.11	11.4	−1.08	12.4	−1.12	13.0
11.7	−1.19	12.0	−1.15	12.4	−1.28	13.0

The chloronitrobenzenes are also reduced to the corresponding hydroxylamines (Table IV). The meta-isomer is the most easily reduced. Below a *p*H of 9 the ortho-isomer is most difficultly reduced with the para-isomer intermediate.

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(4) W. V. McConnell and M. J. Astle, THIS JOURNAL, **65**, 35 (1943).

TABLE IV

HALF-WAVE REDUCTION POTENTIALS (VOLTS *versus* THE S. C. E. CORRECTED FOR *IR* DROP) AND DIFFUSION CURRENTS (MICROAMPERES) FOR 0.001 *M* 50/50 ETHYL ALCOHOL-WATER SOLUTIONS OF CHLORONITROBENZENES

	Ortho		Meta		Para	
*p*H	*E*	*I*	*E*	*I*	*E*	*I*
2.7	−0.64	12.2	−0.53	11.7	−0.59	12.4
4.6	− .75	11.5	− .65	11.5	− .69	12.4
7.4	− .91	11.7	− .86	11.5	− .87	12.2
10.0	−1.01	11.8	− .98	11.5	−1.04	12.4
11.7	−1.07	11.7	−1.05	11.7	−1.15	13.0

Figure 4 shows the effect of *p*H on the reduction potential for the four compounds in which the nitro group is ortho to the second substituent.

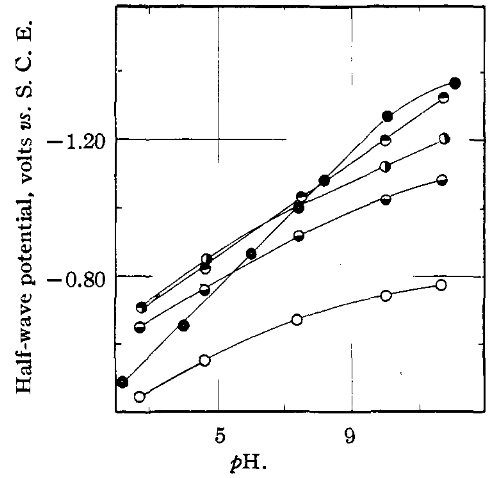

Fig. 4.—Effect of *p*H on the half-wave reduction potentials of the ortho-substituted nitrobenzenes: ○, first wave, dinitrobenzene; ●, nitrobenzoic acid; ◒, nitrochlorobenzene; ◑, nitrotoluene; ◓, second wave, dinitrobenzene.

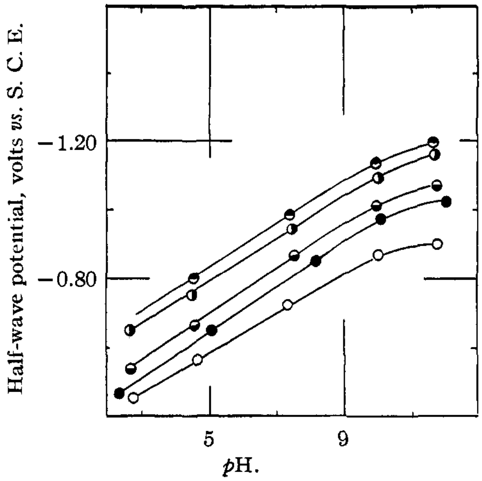

Fig. 5.—Effect of *p*H on half-wave reduction potential of meta-substituted nitrobenzenes: ○, dinitrobenzene, first wave; ●, nitrobenzoic acid; ◒, chloronitrobenzene; ◑, nitrotoluene; ◓ dinitrobenzene, second wave.

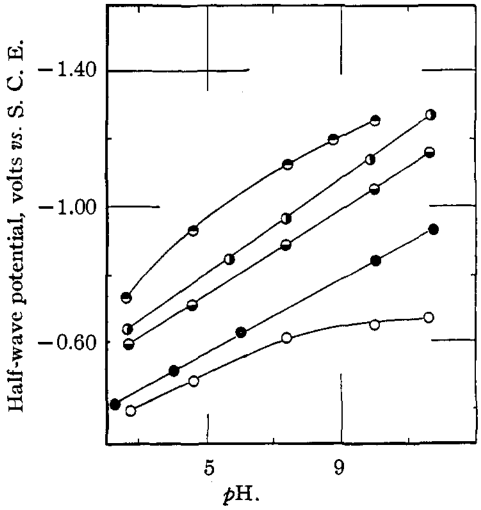

Fig. 6.—Effect of *p*H on the half-wave reduction potentials of para-substituted nitrobenzenes: ○, dinitrobenzene, first wave; ●, nitrobenzoic acid; ◒, nitrochlorobenzene; ◑, nitrotoluene; ◓, dinitrobenzene, second wave.

The same relationship is shown for the meta- and para-isomers in Figs. 5 and 6, respectively. Disregarding for a moment *o*-nitrobenzoic acid (which behaves anomalously), one can observe that in all cases, reduction becomes more difficult with the substituent in the order nitro >, carboxyl >, chloro >, methyl and that this order conforms with Shikata's rule of electronegativity. It would seem, therefore, that the effect of the substituent group is to attract the electrons away from the nitrogen leaving it relatively more positive than a nitrogen in a normal nitro group. This tendency toward placing a positive charge on the nitrogen causes the electrons around the nitrogen to be placed under greater constraint, and as a result the resonance within the nitro group is decreased and the nitro group more readily reduced.

The nitro group and carboxyl group show a $-I$ and a $-M$ effect. Both of these effects would decrease the electron density around the nitrogen thus facilitating the reduction of the nitro group. The $-M$ effect would have a greater effect in the ortho- and para-isomers. Consequently, the meta-isomer should be the most difficultly reduced and this is what was observed except for *o*-nitrobenzoic acid which behaves anomalously in other respects.

Chlorine exhibits a $-I$ and a $+M$ effect while $-CH_3$ exhibits a $+I$ effect. Both of these groups therefore, should increase the electron density around the ortho- and para-positions relative to the meta-positions and therefore the meta-isomers should be more readily reduced. This is what has been observed.

When hydrogen bonding is possible, the ortho substituted nitro compounds are more readily reduced than either the corresponding meta- or para-isomers. This has been observed previously in the investigations of nitro phenolic compounds[5] and was observed in the present work for the second stage reduction of dinitrobenzenes which represents the reduction of nitrophenylhydroxyl amines. The *o*-isomer which may form H-bonds between the nitro and the hydroxylamine group was more readily reduced than the corresponding meta- or para-isomers.

After the completion of the present work, Pearson[6] published the results of his investigations of the polarography of the nitrotoluenes and the dinitrobenzenes. Pearson using a potassium hydrogen phthalate–hydrochloric acid buffer over the *p*H range 2.5–3.8 and a potassium hydrogen phthalate–sodium hydroxide buffer over the *p*H range 4.1–7.4 reported half-wave potentials which were from about 0.25 to 0.35 volt more positive than those found in this Laboratory using different systems. Some of Pearson's work has been repeated and verified in this Laboratory. It seems improbable that the differences in reduction potentials could be accounted for on the basis of large differences in concentrations of the nitrobenzene derivatives used in the two investigations. It seems more probable that the reduction of the nitro group is a function not only of the *p*H but also of the nature of the buffer. This effect is being further investigated.

Pearson obtained a second wave at low *p*H values which corresponded approximately to a two electron process which, when added to the height of the first wave involving a four electron process, represented the complete reduction to the corresponding aniline. This second small wave has also been observed in this Laboratory at a *p*H of 2.7 for all the substituted nitrobenzenes but it was very poorly defined and so was not included in the tabulated data.

Summary

The reduction of the ortho-, meta- and para-isomers of nitrobenzoic acid, dinitrobenzene, chloronitrobenzenes and nitrotoluene from buffered solutions at the dropping mercury cathode have been studied.

Within each group of isomers, the ortho-compound was more difficultly reduced than the para-compound. It is possible to correlate the electronegativity of the second substituent with the reduction potential of the nitro group and to qualitatively determine the effect of position on the degree of the electronegativity of that group.

o-Nitrobenzoic acid does not behave like the other ortho-compounds in that the reduction potential is much more sensitive to differences in *p*H of the solution.

In every case reduction stops with the formation of the corresponding hydroxylamines, with the exception of the dinitrobenzenes and in these cases the complexity of the curves make difficult the determination of the intermediate steps of reduction.

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(5) W. P. Cropper and M. J. Astle, THIS JOURNAL, **65**, 2395 (1943).

(6) J. Pearson, *Trans. Faraday Soc.*, **44**, 683 (1948).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE POLYTECHNIC INSTITUTE OF BROOKLYN AND THE DEPARTMENT OF BIOCHEMISTRY OF THE JEWISH HOSPITAL OF BROOKLYN]

Sulfate Esters as Intermediates in the Formation of 7-Dehydrocholesterol and Dicholesteryl Ether[1]

BY ALBERT E. SOBEL, PHYLLIS S. OWADES[2] AND JOSEPH L. OWADES[3]

A certain amount of evidence has accumulated pertaining to the importance of sulfate esters in the chemistry of steroids in the living organism. At least six different steroid sulfates have been isolated from urine,[4-9] as well as a dehydrosteroid which was postulated as having been formed from a sulfate ester during the course of isolation.[10] A sulfate containing lipid was found in brain.[11] Recently it has been shown that the human kidneys maintain a definite level of sulfate in the blood. The existence of this threshold suggests that the sulfate in the blood, heretofore considered to be simply excretory, plays some metabolic role.[12,13]

Naturally occurring 7-dehydrocholesterol and dicholesteryl ether are found associated with the relatively abundant cholesterol, from which they would seem to be derived. 7β-Hydroxycholesterol, the probable intermediate in the formation of 7-dehydrocholesterol, has also been isolated.

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(1) Presented before the Division of Biological Chemistry of the American Chemical Society at Chicago, Ill., April, 1948.

(2) Abstracted from the thesis submitted to the Faculty of the Graduate School of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the degree of Master of Science in Chemistry.

(3) Abstracted from the dissertation which is being submitted to the Faculty of the Graduate School of the Polytechnic Institute of Brooklyn in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(4) Schachter and Marrian, *Proc. Soc. Exp. Biol. Med.*, **35**, 222 (1936).

(5) Munson, Gallagher and Koch, *J. Biol. Chem.*, **152**, 67 (1944).

(6) Venning, Hoffman and Browne, *ibid.*, **146**, 369 (1942).

(7) Klyne and Marrian, *Biochem. J.*, **39**, XLV (1945).

(8) Klyne, *ibid.*, **40**, 875 (1946).

(9) Paterson and Klyne, *ibid.*, **41**, proc. 2 (1947).

(10) Burrows, Cook, Roeb and Warren, *Biochem. J.*, **31**, 950 (1937).

(11) Levene, *J. Biol. Chem.*, **53**, 614 (1912).

(12) Vars and Gurd, *Am. J. Physiol.*, **151**, 399 (1947).

(13) Letspeich, *ibid.*, **151**, 311 (1947).