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Polarographic Studies of Sulfonamides. I. The Oxidation Products of Sulfanilamide

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Introduction

It was postulated by Mayer, *et al.*,[2a,2b] that sulfonamides are oxidized and that it is these oxidation products which exert bacteriostatic action. A number of investigators tested this theory, notably Bratton, White and Marshall,[3] Sevag, *et al.*,[4,5] and Burton, McLeod, McLeod and Mayr-Hartung.[6] Locke, Main, Mellon and Shinn, in a series of papers,[7] proposed a modification of Mayer's hypothesis. In this modified theory, catalase is inhibited by sulfonamides. The oxidation products of sulfanilamide, such as *p*-hydroxylaminobenzenesulfonamide, decompose with the formation of hydrogen peroxide and azoxybenzenesulfonamide. Since catalase is inhibited, the peroxide formed by the bacteria is able to exercise a bacteriostatic action. According to this theory, the mode of action of sulfonamides is twofold: (A) it inhibits catalase which allows the peroxide, which the cell itself produces, to accumulate, and (B) some oxidation products of sulfonamide also produce hydrogen peroxide by decomposition.

Addition of hydrogen peroxide and other oxidizing agents to sulfonamides has been claimed to potentiate their bacteriostatic efficacy in clinical use, by Goldberger.[8] In the present report, it was decided to investigate the polarographic behavior of some of the oxidation products of sulfanilamide in order to determine whether or not such products can be identified in bacterial media and in body fluids. This method of approach might be a method for testing the oxidation theories. In addition, some bacteriological data on the bacteriostatic effects of *p*-hydroxylaminobenzenesulfonamide are presented.

Experimental

1. Hydroxylaminobenzenesulfonamide Complex.—This compound was prepared by the method of Bratton, White and Marshall.[3] A three-necked flask was set up with a condenser, motor stirrer and a thermometer. The flask contained 2.5 g. of ammonium chloride in 100 ml. of water, and was heated to 65°. Nine grams of *p*-nitrobenzenesulfonamide was ground in a mortar with 7.5 g. of zinc dust. This was added 1 g. at a time to the solution in the flask. Purified hydrogen was passed through the mixture to prevent oxidation. The temperature of the reaction mixture did not exceed 70°. After thirty minutes, the reaction mixture was filtered and the zinc oxide cake washed twice with 25-ml. portions of hot water. Forty-five grams of sodium chloride was added to the filtrate, which was then cooled and the precipitate filtered off. The product was dried in a vacuum desiccator in an ice-chest. The crude product was extracted with three 400-ml. portions of C. P. anhydrous ether and filtered into an equal volume of petroleum ether (b. p. 30–60°). This solution was cooled and filtered and the separated crystals were collected and dried in a vacuum desiccator. The yield was 2.0 g. The white substance melted at 163–164°. The m. p. given by Bratton, *et al.*,[3] was 139.5–140.5°, who quote Mayer as giving 161°, and a solubility in water of 0.12 g. per 100 ml.

The same compound was obtained when the hydroxylamine was prepared by the method of Burton.[9] Hydrogen

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(2)(a) R. L. Mayer, *Bull. Acad. de Med.*, **117**, 727 (1937); (b) R. L. Mayer and C. Oechslin, *Compt. rend.*, **205**, 181 (1937).

(3) A. C. Bratton, H. J. White and E. K. Marshall, Jr., *Proc. Soc. Exptl. Biol. Med.*, **42**, 847 (1939).

(4) M. G. Sevag and M. Shelburne, *J. Bact.*, **43**, 411 (1942).

(5) M. G. Sevag, M. Shelburne and M. Ibsen, *J. Biol. Chem.*, **144**, 711 (1942).

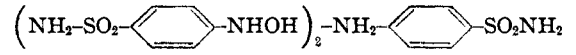
(6) H. Burton, J. W. McLeod, T. S. McLeod and A. Mayr-Hartung, *Brit. J. Exptl. Path.*, **21**, 288 (1940).

(7) A. Locke, E. R. Main and R. R. Mellon, *J. Immunol.*, **36**, 183 (1939); A. Locke, E. R. Main and R. R. Mellon, *Science*, **88**, 620 (1938); E. R. Main, L. E. Shinn and R. R. Mellon, *Proc. Soc. Exptl. Biol. Med.*, **39**, 272 (1938); **42**, 115 (1939); **43**, 593 (1940); L. E. Shinn, E. R. Main and R. R. Mellon, *ibid.*, **39**, 591 (1938); **40**, 640 (1939); **42**, 736 (1939).

(8) H. A. Goldberger, *Am. J. Surg.*, **56**, 353 (1942).

(9) H. Burton, *Chemistry & Industry*, **60**, 449 (1941).

was passed through the reaction mixture. This improved the yield and did not change the melting point, which was again 163–164°. The difference in the melting point of the compound obtained by us and that obtained by Bratton, *et al.*,[3] is explained by the fact that our product is a complex of two molecules of the hydroxylamine compound and one molecule of sulfanilamide, as postulated by Sevag[10]



Anal. Calcd. (for the complex): C, 39.42; H, 4.41. Found: C, 40.14; H, 4.29.

Polarographic studies were made of the hydroxylaminobenzene complex. Both the manual polarograph as described by Kolthoff and Lingane,[11] and the automatic Heyrovsky instrument Model XI,[11] were used. Polarographically, the hydroxylamine was not reducible in acid and in neutral buffer solutions, but was reducible in air-free 0.1 *N* sodium hydroxide. The half-wave potential was found to be −0.68 volt against the saturated calomel electrode (hereafter designated as S. C. E.). The current was proportional to the concentration and from the height of the wave it was inferred that two electrons are involved in the reaction.

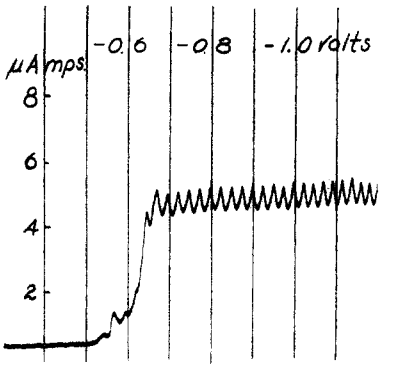


Fig. 1.—Polarogram of hydroxylaminobenzenesulfonamide complex: 0.001 *M* solution in 0.11 *N* NaOH.

It was assumed that in solution the complex dissociates quantitatively into its constituents, hydroxylaminobenzenesulfonamide and sulfanilamide. During this study, it was established that sulfanilamide is not reduced at the dropping mercury electrode, hence the current is due entirely to the hydroxylaminobenzenesulfonamide. Figure 1 represents a polarogram of 0.001 *M* solution of the hydroxylaminobenzenesulfonamide–sulfanilamide complex in 0.11 *N* sodium hydroxide. Table I shows values of the diffusion current.

TABLE I

DIFFUSION CURRENTS OF HYDROXYLAMINEBENZENESULFONAMIDE IN 0.1 *M* SODIUM HYDROXIDE AT 25°

Concentration of hydroxylaminobenzenesulfonamide Mg. per 100 m. of air-free 0.112 *M* NaOH	Concn. (*c*) in millimoles per liter	Diffusion current (i_d) in microamperes	Diffusion current (i_d/c) in microamperes per 0.001 *M* concn.	Half-wave potential ($E_{1/2}$) (volts), against S. C. E.
9.4	0.343	1.48	4.31	−0.62
16.3	.595	2.60	4.37	
25.4	.926	4.00	4.32	
27.7	1.001	4.36	4.33	
46.0	1.680	7.23	4.30	
			Av. 4.33	

(10) M. G. Sevag, THIS JOURNAL, **65**, 110 (1943).

(11) I. M. Kolthoff and J. J. Lingane, *Chem. Rev.*, **24**, 1 (1939).

With the reasonable assumption that two electrons are involved in the reaction according to the equation $RNHOH + 2H^+ + 2e \rightarrow RNH_2 + H_2O$, it is possible to calculate the diffusion coefficient D with the aid of Ilkovic's equation[12]

$i_d = 605nD^{1/2}m^{2/3}t^{1/6}c$, in which
i_d = diffusion current in microamperes
n = number of electrons
m = mg. of mercury flowing out per second
t = drop time in seconds
c = millimoles per liter
D = diffusion coefficient

For our capillary, $m^{2/3}t^{1/6}$ was 1.2 mg.$^{2/3}$ × sec.$^{-1/2}$ at −0.6 volt.

$D^{1/2} = i_d/(605ncm^{2/3}t^{1/6})$
$D = 8.9 \times 10^{-6}$ cm.^{2} sec.$^{-1}$ at 25°

This value of D is a reasonable value for a molecule of this size. The value of D for benzoate ion, which is of similar size, is 8.66×10^{-6} cm.^{2} sec.$^{-1}$ at 25°.[12]

Thus the polarographic method affords a direct way of distinguishing between sulfanilamide and *p*-hydroxylaminobenzenesulfonamide. The latter compound whether prepared by the method of Bratton, *et al.*,[3] or by the method of Burton,[9] gave exactly the same reduction curve. Since the two methods yielded products of identical m. p., it was concluded that the same compound was obtained by both procedures.

As pointed out in the Introduction, Mayer, *et al.*,[2a,b] postulated that hydroxylaminobenzenesulfonamide was responsible for the bacteriostatic action of sulfanilamide, and found that hydroxylaminobenzenesulfonamide was 100 times more bacteriostatic against streptococci than sulfanilamide *in vitro*. Bratton, *et al.*,[3] synthesized the hydroxylaminobenzenesulfonamide, and found that with streptococci *in vitro*, the compound was no more than ten times as active as sulfanilamide, and in some cases considerably less than ten times as active.

Our hydroxylaminobenzenesulfonamide complex was tested against *Escherichia coli*, compared with sulfanilamide in synthetic medium, by a method which has previously been described.[13]

Twenty-five ml. of synthetic medium containing sulfanilamide and the hydroxylaminobenzenesulfonamide complex were inoculated with 0.1 ml. of a twenty-four hour synthetic medium culture of *E. coli*. After twenty-four hours incubation time at 37°, quantitative plate counts were made in quintuplet. The results are seen in Table II.

TABLE II

COMPARISON OF BACTERIOSTATIC ACTION OF HYDROXYLAMINOBENZENESULFONAMIDE COMPLEX (HABS) WITH SULFANILAMIDE (SNA) ON *E. coli* IN SYNTHETIC MEDIUM

Controls with no added drugs, organisms/ml.	SNA, 5 mg. per 100 ml., organisms/ml.	HABS, 5 mg. per 100 ml., organisms/ml.
326×10^6	254×10^5	111×10^5
304×10^6	257×10^5	86×10^5
322×10^6	238×10^5	94×10^5
285×10^6	225×10^5	108×10^5
276×10^6	269×10^5	99×10^5
Av. 305×10^6	249×10^5	99×10^5

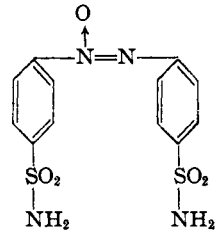
These results show that 5 mg. per 100 ml. of the hydroxylaminobenzenesulfonamide complex is not much more active than an equal concentration of sulfanilamide. Smaller concentrations than 5 mg. per 100 ml. of both sulfanilamide and hydroxylaminobenzenesulfonamide failed to give appreciable inhibition of bacterial growth, under our experimental conditions.

(12) I. M. Kolthoff and J. J. Lingane, "Polarography and Voltammetry, Amperometric Titrations," Interscience Publ., New York, N. Y., 1941.

(13) H. M. Tsuchiya, D. Tenenberg, W. G. Clark and E. A. Strakosch, *Proc. Soc. Exptl. Biol. Med.*, **50**, 262 (1942).

This is in agreement with Bratton, *et al.*,[3] but not with Mayer, *et al.*[1,2] It should be emphasized that the melting point of the hydroxylaminobenzenesulfonamide used in the experiment corresponds to the compound used by Mayer and not to that of Bratton, *et al.*

2. ***p,p'*-Azoxybenzenesulfonamide.**—This compound was prepared from sulfanilamide by oxidation with hydrogen



peroxide. After this work was done, a paper by Carrara and Monzini[14] was published which describes a similar synthesis. In our method, 10 g. of sulfanilamide was dissolved in 800 ml. of water, and 300 ml. of 30% hydrogen peroxide was added to the solution. The temperature of the mixture was held at 80° in a water-bath for six hours. The mixture was cooled and filtered. The precipitate was crystallized twice from a mixture containing 75 ml. of ethanol and 25 ml. of dioxane. The yield was 6.0 g., and the m. p. was 301–302°. Bratton, *et al.*,[3] give 301–302°, and quote Mayer as reporting 300°.

Anal. Calcd. for this compound: C, 40.42; H, 3.394; N, 15.73. Found: C, 40.64; H, 3.367; N, 16.0.

Polarographically, the compound is reducible in acid, neutral and alkaline buffered solutions containing 50% methanol. The compound is insoluble in neutral and acid aqueous solutions. The *p*H was estimated from the concentration or determined with the quinhydrone electrode. The half-wave potential shifts with the *p*H as shown in Table III.

TABLE III

CHANGE IN POLAROGRAPHIC HALF WAVE POTENTIAL ($\pi_{1/2}$) OF AZOXYBENZENESULFONAMIDE WITH *p*H

Medium	*p*H	Diffusion current (i_d) in microamperes, 0.001 *M* soln.	Half-wave potential, ($\pi_{1/2}$) (volts), against S. C. E.
Hydrochloric acid	1.5	4.40	−0.05
Biphthalate	4.2	4.10	− .30
Phosphate	7.2	4.05	− .45
Tetraethylammonium hydroxide	12.5	4.06	− .70

The difference between *p*H 1.5 to 12.5 is 11 *p*H units, and the shift in potential was 0.65 volt, or 0.059 volt per *p*H unit, which is in excellent agreement with the theoretical requirements for a reversible reaction,[11] $R + n(e) + nH^+ \rightarrow RH_n$, where R is the oxidized form, *n* is number of electrons, (e) is the electron and H^+ is the hydrogen ion.

The half wave potential, $\pi_{1/2}$, should be

$$\pi_{1/2} = \pi + \frac{0.0591}{n} \log \frac{R}{RH_n} + 0.591\ pH$$

The polarographic waves showed a steep maximum which could be suppressed with methylene blue or gelatin. These waves are very similar to those obtained with azoxybenzene, although the wave height of the latter is larger than the one obtained with *p,p'*-azoxybenzenesulfonamide. Figure 2 shows a polarogram of 0.0005 *M* azoxybenzenesulfonamide in phosphate buffer at *p*H 7.2 in 50% methanol containing 2×10^{-5} *M* methylene blue as a maximum suppressor. Figure 3 gives the same polarogram in the same medium without the methylene blue.

(14) G. Carrara and G. Monzini, *Chimica e industria*, **23**, 391 (1941).

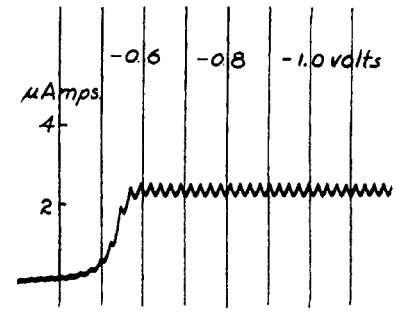


Fig. 2.—Polarogram of azoxybenzenesulfonamide: 0.0005 *M* solution in phosphate buffer at *p*H 7.2 containing 50% methanol and 2×10^{-4} *M* methylene blue.

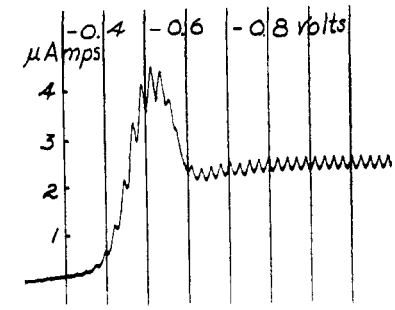
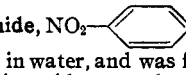


Fig. 3.—Polarogram of azoxybenzenesulfonamide: 0.0005 *M* solution in phosphate buffer at *p*H 7.2 containing 50% methanol, no methylene blue.

Not much is known about the polarographic behavior of azoxy compounds, and further study of the subject is desirable.

3. ***p*-Nitrobenzenesulfonamide,** 

—This compound is soluble in water, and was found to be polarographically reducible in acid, neutral and alkaline aqueous solutions. Table IV shows values of the diffusion current and half wave potential for different *p*H values. Figures 4 and 5 illustrate typical polarograms of 0.001 *M p*-nitrobenzenesulfonamide in neutral and alkaline solutions; they showed a maximum in acid solutions. However, two waves were found in acid medium, as illustrated in Fig. 5. If four electrons were involved (reduction of R-NO_2) then, $D^{1/2} = 7.1/(605 \times 4 \times 1.2)$; $D = 6 \times 10^{-6}$ cm.^{2} sec.$^{-1}$, in neutral and alkaline solutions.

TABLE IV

POLAROGRAPHIC DIFFUSION CURRENTS AND HALF WAVE POTENTIALS OF NITROBENZENESULFONAMIDE AT DIFFERENT *p*H VALUES

Medium	*p*H	Total diffusion current (i_d) in microamperes, of 1×10^{-3} *M* soln.	Half-wave potential ($\pi_{1/2}$) (volts), against S. C. E.
Hydrochloric acid	1.0	10.5	−0.05
Phosphate	7.9	7.1	− .48
Sodium hydroxide	13.0	7.1	− .65

In acid solution the diffusion current for a 0.001 *M* solution of *p*-nitrobenzenesulfonamide was 10.5 microamperes when measured at −0.9 to 1.2 volts against the S. C. E. Apparently six electrons are involved in the complete reduction in acid solution. Such behavior is common to many nitro compounds.

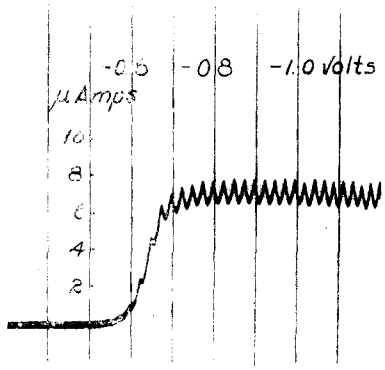


Fig. 4.—Polarogram of nitrobenzenesulfonamide: 0.001 *M* solution in 0.1 *N* NaOH.

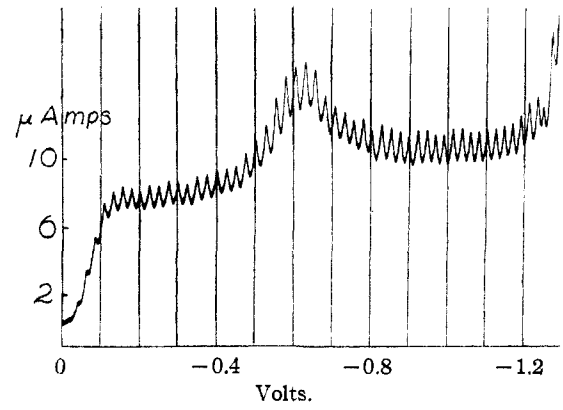


Fig. 5.—Polarogram of nitrobenzenesulfonamide: 0.001 *M* solution in 0.113 *N* HCl.

Summary

1. *p*-Hydroxylaminobenzenesulfonamide, *p,p'*-azoxybenzenesulfonamide, and *p*-nitrobenzenesulfonamide were examined polarographically, and found to be electroreducible at the dropping mercury electrode. The characteristics of the polarographic waves are discussed.

2. A molecular compound composed of two molecules of *p*-hydroxylaminobenzenesulfonamide and one molecule of sulfanilamide, prepared by two different methods, yielded the same melting points and gave exactly the same reduction waves.

3. The bacteriostatic activity of *p*-hydroxylaminobenzenesulfonamide complex against *Escherichia coli in vitro* in a synthetic medium, was not much greater than that of sulfanilamide.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF MANITOBA]

The System Li_2SO_4–H_2O

BY A. N. CAMPBELL

The complete pressure–temperature–concentration diagram of the system Na_2SO_4–H_2O is very well known.[1] The corresponding system Li_2SO_4–H_2O merits attention since the sulfates of sodium and of lithium, alone among the alkali metals, are able to form hydrates, but no systematic study of this has been made. The aim of the present work was to obtain complete information, as far as possible, of these pressure–temperature–concentration relations.

The literature shows that the monohydrate, $Li_2SO_4 \cdot H_2O$, is very stable and usually constitutes the solid phase in equilibrium with solution. The solubility is but little influenced by temperature between 0 and 100°, although the solubility is greater in cold than in hot water.[2] Solubility measurements have been carried down to −16° by Friend.[3] The most recent work on solubility is that of Appleby, Crawford and Gordon.[4]

Nothing is known of the transition temperature for the reaction: $Li_2SO_4 \cdot H_2O \rightleftarrows Li_2SO_4 + H_2O$ (liq.). An approximate calculation of the transition temperature and pressure can be made from the vapor pressure data of the hydrate by Lescoeur[5] and those of the saturated solution by Appleby, Crawford and Gordon.[4] The result of this extrapolation disagrees widely with the value now reported, but this disagreement was to be expected, since the vapor pressure measurements reach only to 110°.

(1) *Cf.*, for example, Findlay and Campbell, "The Phase Rule and Its Applications," Longmans, London, 8th edit., 1938, pp. 179–181, for bibliography.

(2) Seidell, "Solubilities of Inorganic and Organic Compounds," D. Van Nostrand Co., New York, N. Y., 1940, p. 932.

(3) Friend, *J. Chem. Soc.*, 2330 (1929).

(4) Appleby, Crawford and Gordon, *ibid.*, 1665 (1934).

(5) Lescoeur, *Ann. chim. phys.*, **4**, 213 (1896).

Experimental

Determination of the Transition Point.—From the known densities of anhydrous and of hydrated lithium sulfate and assuming that anhydrous lithium sulfate dissolves in water with negligible volume change, the expansion accompanying the transformation: $Li_2SO_4 \cdot H_2O \rightarrow Li_2SO_4$ + satd. solution, is calculated as 8.5%, and this should readily be detected dilatometrically. This calculation neglects the thermal expansion of (superheated) liquid water, which is considerable, so that 8.5% is a lower limit. A dilatometer was prepared from thick-walled hard glass tubing having a stem of coarse capillary. The dilatometer was charged with lithium sulfate monohydrate and aniline as indicator fluid. A thread of pure water was introduced into the top end of the capillary, before sealing off, to give the necessary superincumbent pressure. If this precaution is not taken, the hydrate generates steam at atmospheric pressure at a temperature of 137°, and this may cause the aniline index to break up. From the known dimensions of dilatometer and weight of lithium sulfate monohydrate it was calculated that the transformation would produce a discontinuous linear displacement of the