that given by previous authors, based upon other kinds of experimental work; and the data indicate that the so-called directive influence is less easy to specify than has by many been supposed. For instance, in an equimolal solution of aniline and phenol to which bromine in increasing quantities is added, the bromine goes almost wholly to the phenol until the latter has been converted to monobromophenol, then almost wholly to the aniline until the substance has been converted completely into tribromo-aniline, and finally to change the mono- into tribromophenol.

4. The rate of successive stages of bromination of amino and phenolic compounds is in harmony with the idea that in such cases there is intermediate substitution of bromine in the side chain.

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[CONTRIBUTION FROM THE COLOR LABORATORY OF THE BUREAU OF CHEMISTRY, JOINTLY WITH SOIL FERTILITY INVESTIGATIONS, BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

SPECTROPHOTOMETRIC DETERMINATION OF HYDROGEN-ION CONCENTRATIONS AND OF THE APPARENT DISSOCIATION CONSTANTS OF INDICATORS

IV. 1-NAPHTHOL-2-SODIUM SULFONATE INDOPHENOL[1]

BY WALTER C. HOLMES[2] AND EDWARD F. SNYDER[3]

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The first paper of this series[4] included several representative absorption curves of 1-naphthol-2-sodium sulfonate indophenol in aqueous solutions of various Sörensen (*P*H) values. Although this dye was primarily designed by Clark and Cohen[5] to serve as an oxidation-reduction indicator rather than as an acid-base indicator it is exceptionally suitable, in some respects, for the latter application in connection with spectrophotometric methods. The bands of both color forms occur in spectral regions in which the eye is relatively sensitive to variations in intensity. Any point of transition in the color change, accordingly, can be expressed by means of a ratio R_2 which defines the relative intensities of the two bands. A ratio of this type is sensitive to minimum variation in Sörensen value; furthermore, it is not affected by variation in indicator concentration, owing to its character and to the complete conformity to Beer's law shown by the indicator over a wide range in concentration in aqueous solutions.

Aqueous solutions of the dye are decidedly unstable. The rate of forma-

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[1] Presented before the Dye Section of the American Chemical Society at its Baltimore meeting, April 6-11, 1925.

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[3] Assistant Biochemist, Soil Fertility Investigations, Bureau of Plant Industry.

[4] Holmes, THIS JOURNAL, **46**, 627 (1924).

[5] Clark and Cohen, Pub. Health Repts., **38**, 933-58 (1923).

tion and the character of the decomposition products vary with the Sörensen value of the solution, but the decomposition at any point in the useful range of the indicator is appreciable within 24 hours and relatively considerable within a few days. In absolute alcohol of the ordinary grade, however, the dye is entirely stable. The stock solution of the dye used in the present investigation was prepared with this solvent. Although no particular precaution was taken to protect it from the action of light, no indication of decomposition could be detected over a period of 15 months.

The data of the standardization of the indicator against Sörensen values are recorded in Table I.

Table I

Data for Standardization of 1-Naphthol-2-sodium Sulfonate Indophenol against Sörensen Values

P_H	Indicator concn. Mg. per liter	E at 520$\mu\mu$ Av.	E at 600$\mu\mu$ Av.	R_2
9.69	125	0.526	1.77	(0.297)
9.46	125	.540	1.662	.325
9.28	125	.555	1.59	.349
9.07	150	.670	1.719	.390
8.87	150	.704	1.513	.466
8.67	150	.750	1.252	.600
8.48	200	1.092	1.38	.791
8.27	250	1.40	1.315	1.06 (5)
8.06	250	1.451	0.973	1.49
7.88	250	1.509	.714	2.11
7.67	250	1.535	.514	2.99
7.40	250	1.556	.399	3.90

The buffer solutions employed were the standard 0.05 M boric acid-potassium chloride-sodium hydroxide mixtures of Clark,[6] evaluated electrometrically at 30.0° in an air-bath identical in construction with that described by Clark. The spectrophotometric measurements were made at the same temperature, within a variation of less than 1°. Both series of measurements were made with the equipment and technique described in a former paper.[7]

The approximate indicator concentrations are recorded in the table in terms of milligrams of dye per liter of solution. The concentrations were varied in order to avoid, so far as possible, the necessity of making the absorption measurements at light intensities which were too high or too

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[6] Clark, "The Determination of Hydrogen Ions," Williams and Wilkins Co., Baltimore, 1922.

[7] Holmes and Snyder, This Journal, **47**, 221 (1925). The following corrections are supplied for this and the succeeding paper: On p. 225, Table II, Col. 7, line 5, 0.957 for 0.947. On p. 227, Table I, Col. 6, line 1, 4.75 for 5.75. On p. 228, Table III, beginning with Col. 8:

7:3	8:2	8.5:1.5	9:1
5.04	5.27	5.42	5.62

low for accuracy. As the ratios R_2 are not affected by variation in indicator concentration, no effort was made to avoid minor variation in that particular.

Although the concentration of the indicator was varied, the alcoholic content of the solutions was maintained constant at 5% by adding alcohol in the necessary quantities. The apparent dissociation constant of the indicator is depressed by the presence of alcohol, and it was considered advisable to avoid even minor variation in alcoholic content.

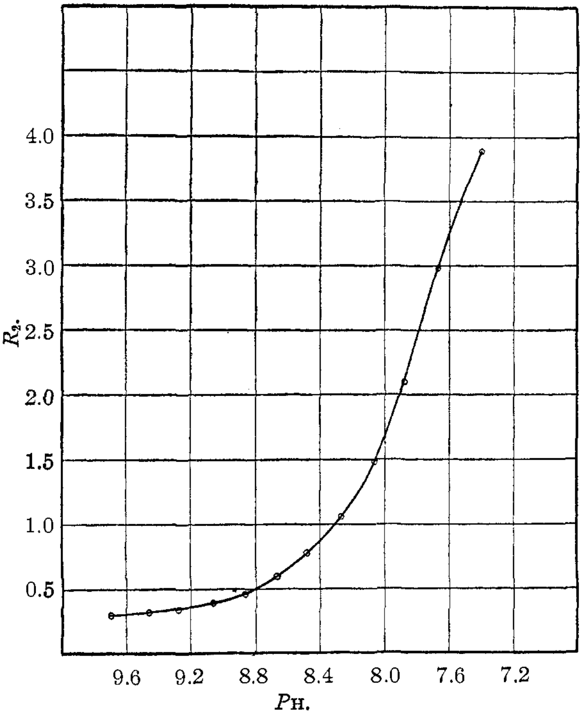

Fig. 1.—1-Naphthol-2-sodium sulfonate indophenol.
$R_2 = \frac{E \text{ at } 520\mu\mu}{E \text{ at } 600\mu\mu}$ in 5% alcohol. Temperature = 30°.

The ratio of the extinction coefficient at 520$\mu\mu$ to that at 600$\mu\mu$ was the spectrophotometric ratio R_2 employed. These wave lengths were chosen in preference to those of 500$\mu\mu$ and 630$\mu\mu$ (the approximate maxima of the bands of the two indicator forms) because it was believed that the consequent gain in visual sensitivity would overbalance the decrease in alteration of ratio value for any given degree of indicator transformation.

The spectrophotometric measurements were made immediately after the preparation of the solutions. This precaution is necessary in any application of the spectrophotometric method with this indicator in the determination of the hydrogen-ion concentration of solutions of unknown Sörensen values. The color and absorption of the decomposition products of the dye differ decidedly from those of the dye itself, and a limited degree of decomposition will invalidate the spectrophotometric ratio. An examination of the solutions immediately after the addition of the indicator gives ample time to determine their R_2 values before the decomposition of the dye can have any measurable effect. A delay of even a few hours should be avoided, however.

The R_2 values obtained are plotted against Sörensen values in Fig. 1.

The curve does not conform to the typical dissociation curve of an indicator. Such conformity is not to be expected with the type of ratio R_2 employed, and it should not be inferred that the dissociation of the indicator is abnormal. On the contrary, the data recorded in the table, supplemented by data obtained over a wider Sörensen range, indicate that the dissociation of the indicator follows the normal course of a monobasic acid.

The apparent dissociation constant of the indicator could be calculated from the recorded data and from other absorption measurements made under conditions insuring practically no dissociation, on the one hand, and practically complete dissociation on the other hand. Preliminary measurements, however, indicated that recourse to Sörensen values necessary to insure practically complete dissociation would be attended by a decrease in stability of the indicator, which might prove a source of minor error. In determining the apparent dissociation constant of the dye, then, it seemed best to employ two solutions of Sörensen values of approximately 6.65 and 10.65, in which the dissociation of the indicator would be 1 and 99%, respectively. The combined absorption of these solutions, contained in absorption cells of equal thickness and containing equal quantitites of indicator, is identical with that of an equivalent total amount of indicator at the mid-dissociation point. The solutions were made up to contain 5% of alcohol. The R_2 value obtained was 0.634, and the corresponding Sörensen value on the curve (and the apparent dissociation constant of the dye) is 8.63. The value obtained by Clark and Cohen[5] with aqueous solutions and by electrometric means was 8.68 at about 20°.

The following data are recorded for use in the drop-ratio method[8] for the determination of hydrogen-ion exponents.

Drop ratio	1:9	2:8	3:7	4:6	5:5	6:4	7:3	8:2	9:1
P_H	(7.68)	8.03	8.26	8.45	8.63	8.81	9.00	9.23	(9.58)

Good agreement has been obtained between the calculated hydrogen-ion exponent values of the drop ratios in this table and standard buffer solutions. In these tests a stock solution of 1 g. of indicator per liter of absolute alcohol was employed, the alcoholic content of the drop-ratio standards

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[8] Gillespie, *Soil Science*, **9**, No. 2, Feb., 1920.

was made approximately 5%, and the buffer solutions for producing the acid and alkaline forms of the indicator had P_H values of about 6.65 and 10.65. The comparison of the solutions to be evaluated with the standards was made as soon as the standards were prepared.

Summary

1. 1-Naphthol-2-sodium sulfonate indophenol is relatively unstable in aqueous solutions but is stable in absolute alcohol.

2. The dissociation of the dye follows the normal course of a monobasic acid.

3. The apparent dissociation constant is 8.63 in aqueous solutions containing 5% of alcohol.

4. Data are supplied for the application of the indicator in the determination of hydrogen-ion exponents by the spectrophotometric and drop-ratio methods.

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[CONTRIBUTION FROM THE ABBOTT LABORATORIES]

ALKYL-ALLYL-BARBITURIC ACIDS[1]

BY ERNEST H. VOLWILER

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Of the numerous types of compounds that have been found to have hypnotic action, none has attained such extensive use as the derivatives of barbituric acid, or malonyl urea. This is due to the relatively greater ease of preparation, higher efficiency and lower toxicity of these compounds. These barbituric acid derivatives, in which the substituent groups are both attached to the 5 carbon atom, were introduced into therapeutic use by Fischer and von Mering[2] who, however, investigated only a limited number of the many possible derivatives. While di-*n*-propylbarbituric acid is twice as active an hypnotic as the diethyl derivative (Barbital) the efficiencies of the di-*iso*butyl and di-*iso*-amyl derivatives show a drop again; from this observation, Fischer apparently concluded that the optimum activity in this homologous series of derivatives resides in the *n*-propyl compound.

The recent availability of higher alcohols has again stimulated investigation in the field, and as a result the series of simple and mixed dialkyl derivatives up to the ethyl-nonyl compound has been rather thoroughly investigated. The 5,5-di-*n*-butyl-barbituric acid[3] was found to be slightly

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[1] Presented at the 69th Meeting of the American Chemical Society at Baltimore, April 10, 1925.

[2] Fischer and von Mering, *Med. Klinik,* **1,** 1327 (1904–5).

[3] Kamm and Volwiler, U. S. pat. 1,331,712 (1920).