



## Keyphrases

Isonicotinoyl hydrazones—synthesis  
Metallic ions—isonicotinoyl hydrazones—  
complexes

Antitubercular activity—cupric, cobalt ions  
effect

## Potential Diazo Reagents for Colorimetric Determination

By TIBOR URBÁNYI and JOSEPH A. MOLLICA

The diazonium derivatives of 27 substituted aromatic amines were investigated as reagents for colorimetric determination. The phenolic moiety used in the study was the estrogen, estradiol. Their applicability was determined on the basis of speed, sensitivity, color stability, and reproducibility of the coupling reaction. Using these criteria, the diazotization product of 4-amino-6-chloro-*m*-benzenedisulfonamide appeared to be the most suitable colorimetric reagent for the compounds containing a phenolic hydroxyl group.

**A** RAPID AND SENSITIVE method for the determination of estrogens is based on their ability to couple with diazotized amines. This communication considers the results of investigations regarding the suitability of various substituted aromatic amines as analytical reagents for compounds having coupling capability. Under the experimental condition employed, 4-amino-6-chloro-*m*-benzenedisulfonamide appeared to be the most promising reagent. The mechanism for the formation of its diazonium compound and its application to the analysis of several estrogens was reported previously (1, 2).

The following criteria for suitability were established: the coupling reaction should be rapid at room temperature, the color produced should be relatively stable, and the method should be sensitive. Since speed, sensitivity, and reproducibility were the objectives, the same reaction conditions were employed for all amines. Although these conditions were not optimum for all amines tested, this procedure was selected since the goal was to find suitable analytical reagents and not to investigate the reaction for each amine.

### EXPERIMENTAL

A Beckman DU spectrophotometer was used to determine the absorbance values, and a Cary model 11 spectrophotometer was used to record the absorption spectra.

All compounds were of reagent grade quality and were used without further purification.

**Reagents and Solutions**—Aromatic amines: a  $7 \times 10^{-3}$  *M* solution of the amine was prepared in either methanol or 1 *N* hydrochloric acid. Sodium nitrite, 1% aqueous solution; hydrochloric acid, 1 *N*; sodium acetate 2 *N*; sodium hydroxide 0.1 *N* and 1 *N*. The estradiol solution was prepared as follows: about 50 mg. of estradiol, accurately weighed, was dissolved in 50 ml. of methanol and then this solu-

tion was diluted 10-fold with 0.1 *N* sodium hydroxide; concentration approximately 0.1 mg./ml.

**Procedure**—Into a 10-ml. volumetric flask were pipeted 1 ml. of the aromatic amine solution, 1 ml. of sodium nitrite solution, and 1 ml. of 1 *N* hydrochloric acid. The solution was mixed well and allowed to stand for 1 to 2 min. Then, 1 ml. of estradiol solution and 2 ml. of sodium acetate solution were added, mixed, and the resulting solution allowed to stand for exactly 6 min. The contents of the flask were diluted to volume with 1 *N* sodium hydroxide solution. A blank solution was prepared in the same manner except that 1 ml. of 0.1 *N* sodium hydroxide was used in place of the estradiol solution. The absorbance of the sample solution was measured against the blank solution at the wavelength of the absorption maximum in 1-cm. cells.



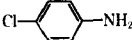


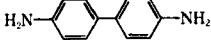
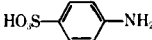

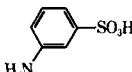
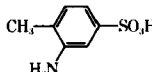
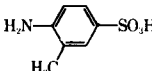
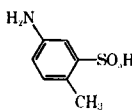
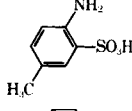
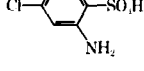
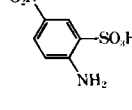
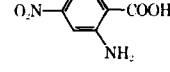
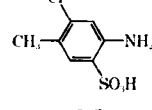
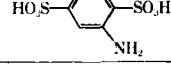
### DISCUSSION

The coupled products yielded absorption spectra with maxima in the range 450–550 *mμ*. The spectra were all similar to the spectrum of 4-amino-6-chloro-*m*-benzenedisulfonamide and estradiol which was published previously (2). The apparent molar absorptivity values in Table I were obtained by dividing the absorbance of the coupled solution, measured at the wavelength of absorption maximum, by the concentration of the amine employed. The molar absorptivity values reported are only apparent values since for many of the compounds investigated, the absorbance continued to increase after the 6-min. reaction time. This is due to either incomplete coupling and/or side reactions of the diazotized amine (3, 4). As stated in the introduction, the purpose of this study was not to investigate the mechanism of reaction, but to find a suitable analytical reagent. However, some conclusions regarding the effect of substituent groups on color intensity can be drawn. Comparisons of the molar absorptivity values indicate that substitution strongly influences the intensity of the coupled product, *e.g.*, 3 and 4, 13 and 15, 23 and 24; not

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TABLE I—SUBSTITUTED AROMATIC AMINES FOR DETERMINATION OF ESTROGENS

Compound	Structural Formula	Initial Solvent	Wavelength, Abs. Max.	Apparent Molar Absorptivity <sup>a</sup>
(1) Aniline		MeOH	NC	NC
(2) <i>o</i> -Chloroaniline		MeOH	465 mμ	2,040
(3) <i>p</i> -Chloroaniline		MeOH	465 mμ	1,500
(4) <i>o</i> -Nitroaniline		MeOH	500 mμ	3,130
(5) <i>p</i> -Nitroaniline		MeOH	525 mμ	2,940
(6) Benzidine		MeOH	NC	NC
(7) Sulfanilic acid		1 N HCl	490 mμ	2,380
(8) <i>o</i> -Aminobenzenesulfonic acid		1 N HCl	NC	NC
(9) Metanilic acid		1 N HCl	490 mμ	2,180
(10) 3-Amino- <i>p</i> -toluenesulfonic acid		1 N HCl	450 mμ	680
(11) 4-Amino- <i>m</i> -toluenesulfonic acid		1 N HCl	450 mμ	680
(12) 5-Amino- <i>o</i> -toluenesulfonic acid		1 N HCl	510 mμ	730
(13) 6-Amino- <i>m</i> -toluenesulfonic acid		1 N HCl	NC	NC
(14) 2-Amino-4-chloro-benzenesulfonic acid		MeOH	NC	NC
(15) 2-Amino-5-nitro-benzenesulfonic acid		MeOH	537 mμ	8,210
(16) 2-Amino-4-nitro-benzoic acid		MeOH	450 mμ	708
(17) 6-Amino-4-chloro- <i>m</i> -toluenesulfonic acid		MeOH	NC	NC
(18) 2-Amino- <i>p</i> -benzenedisulfonic acid		1 N HCl	475 mμ	1,020

(Continued on next page.)

TABLE I (Continued)

Compound	Structural Formula	Initial Solvent	Wavelength, Abs. Max.	Apparent Molar Absorptivity <sup>a</sup>
(19) 4-Amino-6-chloro- <i>m</i> -benzene-disulfonic acid		MeOH	500 mμ	6,560
(20) Sulfanilamide		MeOH	487 mμ	4,440
(21) 2-Amino-4-chlorobenzenesulfonamide		MeOH	475 mμ	1,630
(22) 4-Amino- <i>m</i> -benzenedisulfonamide		MeOH	480 mμ	4,134
(23) 4-Amino-6-chloro- <i>m</i> -benzenedisulfonamide		MeOH	500 mμ	8,170
(24) 4-Amino-6-chloro- <i>N</i> <sup>1</sup> , <i>N</i> <sup>3</sup> -dimethyl- <i>m</i> -benzenedisulfonamide		MeOH	NC	NC
(25) 4-Amino-5-chloro- <i>m</i> -benzenedisulfonamide		MeOH	470 mμ	1,768
(26) 2-Amino-1-naphthalenesulfonic acid		MeOH	NC	NC
(27) 4-Amino-1-naphthalenesulfonic acid		MeOH	NC	NC

<sup>a</sup> Determined after a coupling reaction time of 6 min. in pH 5 acetate buffer. NC = No color.

only the substituent group, but also its position relative to the amine function affects the intensity, e.g., 7 and 8, 15 and 16, 23 and 25. In general, the most sensitive amines were those possessing electron-withdrawing groups *ortho* and/or *para* to the amine function. The relative effects of various substituents on the intensity of the coupled product are in general agreement with the observations of Zollinger for various azo dyes (5).

The authors recognize that without more kinetic data the interpretation of the above-mentioned effects is limited. The data are consistent with what one could predict based on substituent effects on spectral properties or reaction rates.

Taking this into consideration, it is still felt that the data in Table I will be of value to other workers who may be desirous of utilizing a coupling procedure. The amines tested should react in an analogous manner regardless of the phenolic moiety. With perhaps slight adjustment of experimental conditions

to suit particular cases, amines numbered 15, 19, 20, 22, and 23 should be more suitable as diazonium reagents than those numbered 1, 10, 11, 12, 16, 18, and 21.

#### SUMMARY

1. Twenty-seven aromatic amines have been investigated as potential diazo reagents for the colorimetric determination of phenolic compounds. The criteria for suitability established were speed, sensitivity, and reproducibility.

2. The compound 4-amino-6-chloro-*m*-benzenedisulfonamide was considered the most suitable based on the criteria established. Although compound 15 has a greater apparent molar absorptivity, it was not selected as the most suitable since the color produced was not as stable as that produced with compound 23.

3. Amines with electron withdrawing groups

*ortho* and/or *para* to the amine group possess the greatest potential.

## REFERENCES

- (1) Urbányi, T., Pagliery, M., and Rehm, C. R., *Anal. Chem.*, **39**, 1656(1967).
- (2) Urbányi, T., and Rehm, C. R., *J. Pharm. Sci.*, **55**, 501(1966).
- (3) Kuhn, R., and Löw, I., *Berichte Deutschen Chem. Ges.*, **72**, 1453(1939).
- (4) Brown, E. B., Bina, A. F., and Thomas, J. M., *J. Biol. Chem.*, **158**, 455(1945).
- (5) Zollinger, H., "Azo and Diazo Chemistry," Interscience Publishers, Inc., New York, N. Y., 1961.



## Keyphrases

Reagents—colorimetric analysis  
 Amines, substituted—diazonium derivatives  
 4-Amino-6-chloro-*m*-benzene-disulfonamide—  
 estradiol analysis  
 Apparent molar absorptivity values—diazonium reagents  
 Colorimetric analysis—spectrophotometry

## Potentiometric Determination of Fluoride Ion in Toothpastes by a Specific Ion Activity Electrode

By NORMAN SHANE and DENNIS MIELE

A simple, specific potentiometric method is described for the assay of toothpastes for free fluoride ion in concentrations of about 0.1 percent, equivalent to 0.22 percent sodium fluoride or 0.4 percent stannous fluoride. The ionic fluoride content of the toothpastes is determined by relating the potential obtained with a fluoride electrode in an aqueous dispersion of toothpaste to a calibration curve constructed by adding known scalar amounts of fluoride to a blank toothpaste formulation.

THE DETERMINATION of fluoride ion concentration in toothpastes has ordinarily required steam distillation as a separation procedure and titration by thorium nitrate for the fluoride ion using a colored dye as an indicator. The classical Willard-Winter distillation (1), as modified by Grimaldi *et al.* (2), Singer and Armstrong (3), and Wade and Yamamura (4), has been the basis for most analytical procedures. Following distillation, spectrophotometric methods utilizing the blue complex formed between the fluoride ion and the cerium (III) salt of 3-amino-methyl-alizarin-*N,N*-acetic acid have been employed by Bellack *et al.* (5). Also, Harley and Revinson (6) and Calkins (7) have used high-frequency titration techniques, and other methods are described in review articles by Fishman *et al.* (8). Frant (9) explained the theory and operation of the fluoride ion electrode. In contrast to the potentiometric method as explained in this paper, Lingane (10) showed a titration method for fluoride ion employing the fluoride electrode.

The specific method for free fluoride ion described in this paper requires no separation steps. Fluoride ion is detected in toothpastes in the range of 0.1% F<sup>-</sup> ion equivalent to 0.22% NaF and 0.40% SnF<sub>2</sub>. An important consideration in this analysis is that the fluoride must be present as a free ion. The fluoride ion activity electrode model 94-09, developed by Orion Research, Inc., was used as easily as a conventional pH electrode with an Orion digital meter, model 801. A standard glass sleeve calomel reference electrode was employed in conjunction with the fluoride electrode. The electrodes were immersed in an aqueous mixture of toothpaste

so as to measure the activity of ionized (free) fluoride in terms of concentration. By use of an appropriate calibration curve, the concentrations of F<sup>-</sup> ion from NaF and SnF<sub>2</sub> were determined.

## EXPERIMENTAL

**Apparatus and Reagents**—Fluoride electrode, model 94-09, obtained from Orion Research, Inc., Cambridge, Mass.; Orion Research meter 801; Beckman calomel electrode (with glass sleeve); 50-ml. Nalgene centrifuge tube; 100-ml. volumetric flask; 150-ml. beaker; Sargent magnetic stirrer.

**Stirrer**—Made from an electric toothbrush with a special made paddle. This paddle is a 10.1-cm. aluminum shaft and has a 3.2 mm. diameter. Two fins, each 2.5 cm. long and 1.9 cm. wide, are located at the stirring end.

**NaF Stock Solution for Calibration Curve**—180 mg. NaF/100 ml. aqueous solution (0.18% NaF),<sup>1</sup> 200 mg. NaF/100 ml. aqueous solution (0.20% NaF), 220 mg. NaF/100 ml. aqueous solution (0.22% NaF), 240 mg. NaF/100 ml. aqueous solution (0.24% NaF), 260 mg. NaF/100 ml. aqueous solution (0.26% NaF).

**SnF<sub>2</sub> Stock Solution for Calibration Curve**—To each solution is added 400 mg. of malic acid as a stabilizer. Deoxygenated water is used so as to further prevent oxidation of the stannous ion. 320 mg. SnF<sub>2</sub>/100 ml. aqueous solution (0.32% SnF<sub>2</sub>),<sup>2</sup> 360 mg. SnF<sub>2</sub>/100 ml. aqueous solution (0.36% SnF<sub>2</sub>), 400 mg. SnF<sub>2</sub>/100 ml. aqueous solution (0.40% SnF<sub>2</sub>), 440 mg. SnF<sub>2</sub>/100 ml. aqueous solution (0.44% SnF<sub>2</sub>), 480 mg. SnF<sub>2</sub>/100 ml. aqueous solution (0.48% SnF<sub>2</sub>).

## PROCEDURE

**For NaF and SnF<sub>2</sub> Calibration**—Weigh approxi-

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<sup>1</sup> 1 ml. of these solutions represent 0.18%, 0.20%, etc., of NaF based on 1 g. of paste.

<sup>2</sup> 1 ml. of these solutions represent 0.32%, 0.36%, etc., of SnF<sub>2</sub> based on 1 g. of paste.