

hydroxide. After forty-five minutes at room temperature, the solution was neutralized with *N* hydrochloric acid and the acetone removed by distillation under reduced pressure. The water insoluble sirup was taken into ethyl acetate, and the solution dried and concentrated. The resulting sirup was hydrogenated in 60 cc. of methanol containing 4 cc. of acetic acid and 4 cc. of water with palladium catalyst. The reduction was complete after forty-eight hours. The catalyst was removed by filtration, the filtrate concentrated under reduced pressure to a sirup from which the acetic acid and water were removed by repeated concentration with anhydrous methanol. The product was crystallized from methanol-ether and dried at 75° *in vacuo*: yield 2.60 g.;  $[\alpha]^{21}_D - 17.0^\circ$  (*c* 5, water). Fischer<sup>12</sup> found  $[\alpha]^{20}_D - 17.2$  and  $-16.8^\circ$ .

**Carbobenzoxy- $\beta$ -alanyl-L-leucinamide.**—Carbobenzoxy- $\beta$ -alanine,<sup>18</sup> 6.65 g., in anhydrous ethyl ether, 25 cc., was converted to the acid chloride as previously described<sup>13</sup> and was added to an ether solution of L-leucine methyl ester prepared from 5.9 g. of the hydrochloride. After a few minutes at 0°, 3.5 g. of potassium bicarbonate in water was added and the mixture shaken frequently for an hour during which time the mixture was allowed to come to room temperature. The ether layer was separated and washed with dilute hydrochloric acid and with water. The solution was dried over sodium sulfate and then concentrated under reduced pressure to a sirup. The sirupy product was converted to the amide as described above; needles, yield 5.70 g., m. p. 172–173°. The amide was recrystallized from ethanol-water, m. p. 174–175°.

*Anal.* Calcd. for  $C_{17}H_{25}O_4N_3$ : C, 60.9; H, 7.5; N, 12.5. Found: C, 61.0; H, 7.2; N, 12.5.

(13) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

**$\beta$ -Alanyl-L-leucinamide Hydrochloride.**—This was prepared by hydrogenation of 2.50 g. of carbobenzoxy- $\beta$ -alanyl-L-leucinamide. The reduction was complete in three hours and the catalyst was removed by filtration. The filtrate was concentrated under reduced pressure to a sirup and this procedure repeated several times after the addition of successive small portions of ethanol. Since the product failed to crystallize as the acetate, the sirup was dissolved in 30 cc. of ethanol and 1 cc. of concentrated hydrochloric acid was added. The compound crystallized as the hydrochloride upon the addition of ether as plates; yield after drying *in vacuo* over sulfuric acid at 25°, 1.55 g.; m. p. 120° with evolution of gas; hygroscopic,  $[\alpha]^{26}_D - 17^\circ$  (*c* 2, water).

*Anal.* Calcd. for  $C_9H_{20}O_2N_3Cl$ : C, 45.5; H, 8.5; N, 17.7. Found: C, 45.3; H, 8.6; N, 17.6.

**Acknowledgment.**—This investigation was aided by grants from the United States Public Health Service.

### Summary

1. The preparation and properties of some dipeptides and dipeptide derivatives containing L-leucine, D- and L- $\alpha$ -alanine and  $\beta$ -alanine are described.
2. L-Leucyl-D-alanine and L-leucyl-L-alanine have been synthesized from L-leucine and racemic alanine.
3. The enzymatic preparation of carbobenzoxy-D-alanine from carbobenzoxy-DL-alanine is described.

SALT LAKE CITY, UTAH

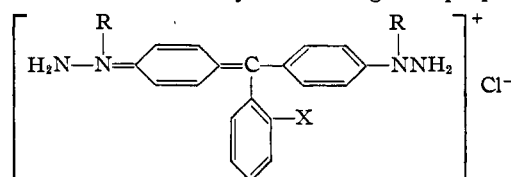
RECEIVED APRIL 30, 1949

[CONTRIBUTION FROM THE BALLISTIC RESEARCH LABORATORIES

## Triphenylmethane Dyes Containing the Hydrazine Group and Their Condensation Products with Aldehydes

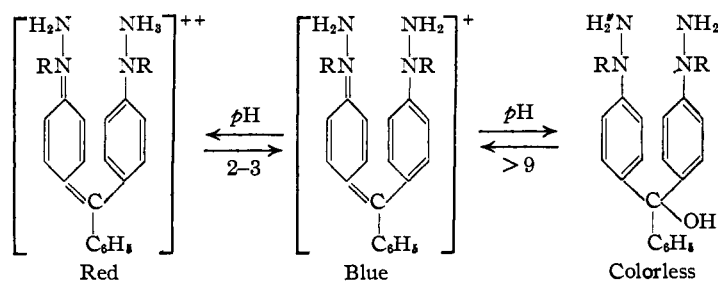
BY LESTER P. KUHN AND LOUIS DEANGELIS

In order to obtain an improved reagent for the determination of aldehydes we sought to prepare a



- I. X = H, R = H  
 II. X = H, R = CH<sub>3</sub>  
 III. X = SO<sub>3</sub><sup>-</sup>, R = CH<sub>3</sub>

dye which contains a functional group capable of reacting readily with aldehydes and which as a result of its reaction would change color. Schwarzenbach<sup>1</sup> prepared *N,N'* substituted phenylhydrazine sulfonylphthalein dyes and showed that they are deeply colored stable compounds quite similar in properties to the aniline sulfonylphthaleins. More recently *p,p'*-dihydrazinotriphenylmethyl chloride,



(1) Schwarzenbach, *Helv. Chim. Acta*, **20**, 498 (1937).

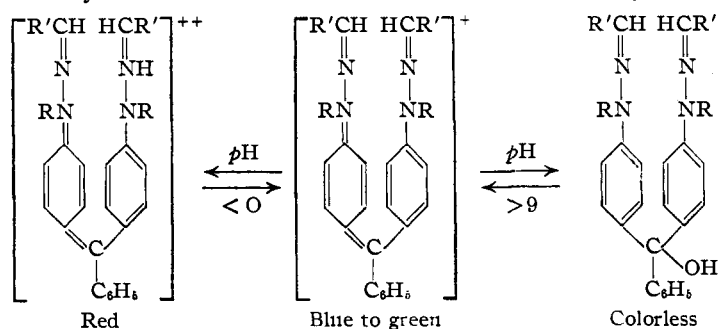
(2) Ciusa and Ottolingo, *Gazz. chim. ital.*, **76**, 171 (1945); *C. A.*, **41**, 4187 (1947).

I, and its *N*-methyl derivative, II, have been prepared.<sup>2</sup> It was shown that these dyes react readily with aldehydes, but not with ketones, and that the color of the dye solution changes from red to blue or to green. In the present work the preparation of I and II has been repeated, and the new dye III has been prepared. An attempt has been made to further evaluate these compounds as reagents for the determination of aldehydes and also to explain the color changes which are observed.

The dyes I, II and III can exist in several dif-

ferent states depending upon the pH of the solution in the manner shown here.

The hydrazones show a similar behavior



In the hydrazone series the conversion of the singly charged cation to the doubly charged cation requires a relatively concentrated solution of sulfuric or hydrochloric acids whereas in the hydrazine series the change occurs at a  $pH$  of 2-3. This difference which is due to the stronger basicity of the hydrazine group as compared with the hydrazone makes possible the use of these dyes as extremely good reagents for aldehydes. Ciusa and Ottolino<sup>2</sup> described the hydrazine dye as red and the hydrazone dye as blue or green and did not mention the fact that the color of these compounds depends upon the solution  $pH$ . Apparently they happened to be working in the  $pH$  range in which the hydrazine dye is stable in its red form and the hydrazone in its blue form.

A solution of any of the hydrazine dyes may be prepared in either water, methanol, or mixtures of the two and acidified to the point where the red color is obtained. Upon the addition of a drop of solution containing an aldehyde a color change is obtained within a minute. With aliphatic alde-

hydes the color becomes blue and with aromatic aldehydes it becomes green due to the formation of the hydrazone dye. The results obtained with a number of different aldehydes are given in Table I. Interestingly enough, ketones do not react under the conditions employed except the very readily enolizable ketone, ethyl acetoacetate. Sugars and chloral hydrate were the only aldehydes which did not give a positive test. With this reagent we have been able to detect 2 parts of benzaldehyde in a million parts of methanol.

Figures 1, 2 and 3 show the absorption curves for dyes I, II and III, respectively, in the red and blue forms and also the curves for the hydrazones formed from formaldehyde, acetaldehyde and benzaldehyde.

For the quantitative determination of aldehydes dye III should be used because its hydrazones are more soluble than the hydrazones of dyes I and II. A calibration curve is first prepared by making up solutions of known concentrations of the aldehyde to be determined and excess dye and then obtaining the optical density as a function of concentration. Figure 4 shows the calibration curves for acetaldehyde and benzaldehyde. The latter is a straight line but the former is slightly concave downward because the unreacted hydrazine dye has an appreciable absorption at the wave length where the absorption maximum of the acetaldehyde hydrazone occurs. The concentration of aldehyde in the solution to be analyzed can be obtained by comparing the intensity of color which it yields upon reaction with the reagent with the calibration curve, if the experimental conditions are kept constant. It is beyond the scope of this paper to make a complete evaluation of this reagent

TABLE I

## EFFECT OF VARIOUS SUBSTANCES UPON DYES

Compound	Color produced
Formaldehyde	Blue
Acetaldehyde	Blue
Benzaldehyde	Green
Glyoxal	Blue
Furfural	Green
Crotonaldehyde	Green
Chloral hydrate	No change
Anisaldehyde	Green
<i>p</i> -Nitrobenzaldehyde	Green
Glucose	No change
Fenchone	No change <sup>a</sup>
Acetone	Deeper red <sup>b</sup>
Acetophenone	Deeper red <sup>b</sup>
Paraldehyde	Blue
Acetonyl acetone	No change <sup>b</sup>
Methylglyoxal	Purple
Piperonal	Green
Ethyl acetoacetate	Blue
Cinnamaldehyde	Green
<i>p</i> -Dimethylaminobenzaldehyde	Green

<sup>a</sup> Turns blue after standing for ten minutes. <sup>b</sup> Turns purple after standing for ten minutes.

TABLE II

ABSORPTION MAXIMA OF DYES OF FORM R=D-R<sup>a,b</sup>

R	Triphenylmethane dyes <sup>a</sup>	Sulfonphthalein dyes <sup>b</sup>
NH <sub>2</sub>	562	576
NHNH <sub>2</sub>	580	
NHCH <sub>3</sub>		604
N(CH <sub>3</sub> ) <sub>2</sub>	618	
NCH <sub>2</sub> NH <sub>2</sub>	590	610
NHN=CH <sub>2</sub>	615	
NCH <sub>2</sub> N=CH <sub>2</sub>	625	635
NHN=CHCH <sub>3</sub>	640	
NCH <sub>2</sub> N=CHCH <sub>3</sub>	645	660
NHN=CHC <sub>6</sub> H <sub>5</sub>	685	
NCH <sub>2</sub> N=CHC <sub>6</sub> H <sub>5</sub>	700	705
NHN=CHC <sub>6</sub> H <sub>4</sub> NO <sub>2</sub> - <i>p</i>	680	
NCH <sub>2</sub> N=CHC <sub>6</sub> H <sub>4</sub> NO <sub>2</sub> - <i>p</i>	695	700
NHN=CHC <sub>6</sub> H <sub>4</sub> OCH <sub>3</sub> - <i>p</i>	705	
NCH <sub>2</sub> N=CHC <sub>6</sub> H <sub>4</sub> OCH <sub>3</sub> - <i>p</i>	730	730
NHN=CH-CH=CH-C <sub>6</sub> H <sub>5</sub>	710	
NCH <sub>2</sub> N=CH-CH=CH-C <sub>6</sub> H <sub>5</sub>	735	735

<sup>a</sup> D = C<sub>6</sub>H<sub>4</sub>C(C<sub>6</sub>H<sub>5</sub>)C<sub>6</sub>H<sub>5</sub>. <sup>b</sup> D = C<sub>6</sub>H<sub>4</sub>C(C<sub>6</sub>H<sub>4</sub>SO<sub>3</sub>)C<sub>6</sub>H<sub>4</sub>.

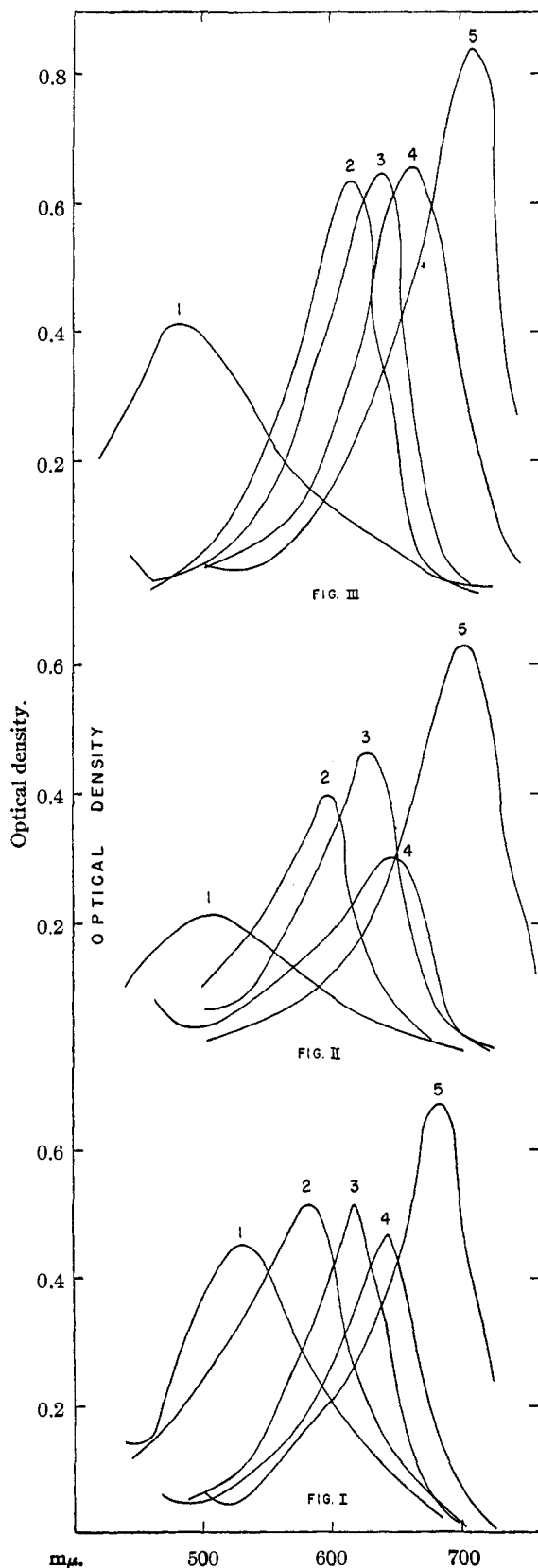


Fig. 3.—Absorption spectrum of III in methanol-water: 1, pH < 2; 2, pH 5; 3, formaldehyde added pH < 2; 4, acetaldehyde added pH < 2; 5, benzaldehyde added pH < 2.

Fig. 2.—Absorption spectrum of II in methanol-water: 1, pH < 2; 2, pH 5; 3, formaldehyde added pH < 2; 4, acetaldehyde added pH < 2; 5, benzaldehyde added pH < 2.

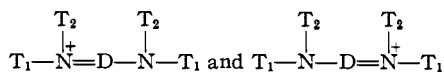
Fig. 1.—Absorption spectrum of I in methanol-water: 1, pH < 2; 2, pH 5; 3, formaldehyde added pH < 2; 4, acetaldehyde added pH < 2; 5, benzaldehyde added pH < 2.

for quantitative determinations but it is rather our intention to indicate the possibility of its use.

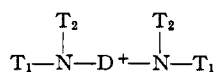
The absorption maxima of the various dyes are given in Table II. The following changes in the dye molecule result in a deepening of the color (bathochromic effect): replacement of the amino group of the diaminotriphenylmethane dye with the hydrazine group, replacement of the hydrogen atom on the nitrogen with methyl, conversion of the hydrazine dye to a hydrazone. The position of the absorption maximum of the hydrazone dyes derived from aldehydes of the general formula, RCHO, is displaced toward the longer wave lengths as R goes through the series H, CH<sub>3</sub>, *p*-nitrophenyl, phenyl, *p*-methoxyphenyl, C<sub>6</sub>H<sub>5</sub>-CHCH.

A number of interesting relationships between the constitution of cyanine dyes and their color have been deduced by Brooker.<sup>3</sup> These relationships have been extended to the dyes of the triphenylmethane series by Branch and co-workers.<sup>4</sup>

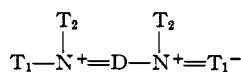
It is of interest to apply these relationships to these new dyes. The dyes in their deeply colored state have a high degree of resonance and it is possible to picture the various structures which contribute to the actual state of the molecule using the symbolism devised by Branch.<sup>4</sup> Let us represent the dye by the symbol T<sub>1</sub>T<sub>2</sub>N—D—N—T<sub>1</sub>T<sub>2</sub>, where D is C<sub>6</sub>H<sub>4</sub>C(C<sub>6</sub>H<sub>5</sub>)-C<sub>6</sub>H<sub>4</sub>, the N's are the auxochromic nitrogen atoms and T<sub>1</sub> and T<sub>2</sub> are the terminal groups bound to these nitrogen atoms. Thus for dye I, T<sub>1</sub> is H and T<sub>2</sub> is NH<sub>2</sub>. The various structures contributing to the actual structure of the molecule are



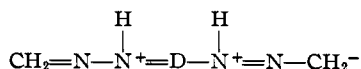
which are the A structures



which is the P structure and



which is the T structure. Contributions from the T structures are possible only if T<sub>1</sub> or T<sub>2</sub> contains a double bond. It may be illustrated by the formaldehyde hydrazone of dye I



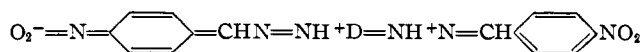
Constitutive features which increase the stability of structures A and T have a bathochromic effect upon the dye. The stability of the A structure depends upon the ability of the nitrogen to contribute its unshared electrons to the resonating system and thereby becoming positively charged.

(3) Brooker, *Rev. Modern Phys.*, **14**, 275 (1942).

(4) Tolbert, Branch and Berlenbach, *THIS JOURNAL*, **67**, 887 (1945); (b) Branch, Tolbert and Lowe, *ibid.*, **67**, 1653 (1945).

This ability depends upon the nature of the substituents on the nitrogen atom, the more electronegative the substituent the less will be the tendency of the nitrogen to donate its unpaired electrons. Since hydrogen is more electronegative than NH<sub>2</sub> which is more electronegative than CH<sub>3</sub>, it is in agreement with theory that the hydrazino-triphenylmethane dye absorbs at longer wave length than the amino dye but at slightly shorter wave length than the methylamino dye.

Conversion of the hydrazine dyes to the hydrazone dyes with an aldehyde, RCHO, reduces the basicity of the auxochromic nitrogen and hence reduces the stability of the A structure which should result in a hypsochromic effect. However, it makes possible the existence of the T structures and the net result is a deepening of the color. When R is aromatic the colors are deeper than when R is aliphatic because the polarizability of the benzene ring makes the T structures more stable. The introduction of the electron attracting nitro group into the benzene ring of R would be expected to produce a bathochromic effect since it stabilizes the T structure



Similarly the methoxy group should produce a hypsochromic effect since it tends to yield electrons and become positively charged, and hence reduces the stability of the T structure. Since the expected effects are opposite to those which are actually observed it seems likely that other resonance forms must be considered which make significant contributions to the actual state of the molecule and which influence the color. For the

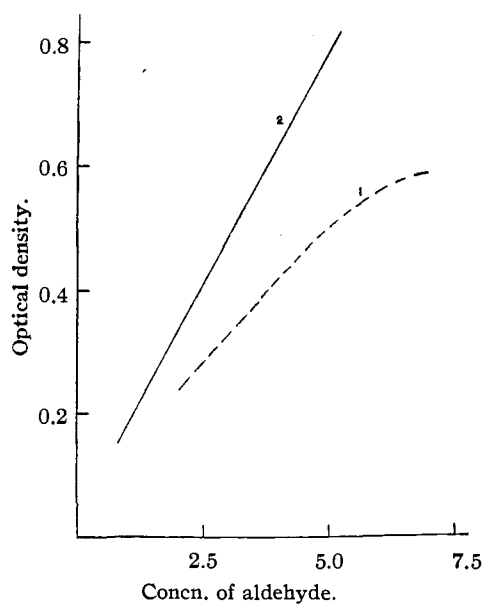
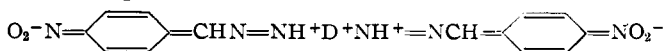
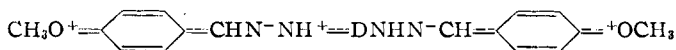


Fig. 4.—Calibration curve for the determination of aldehydes with III pH < 2: 1, acetaldehyde; 2, benzaldehyde.

nitro compound the structure



which is similar to the P structure may account for the observed hypsochromic effect. The methoxy group would stabilize the form

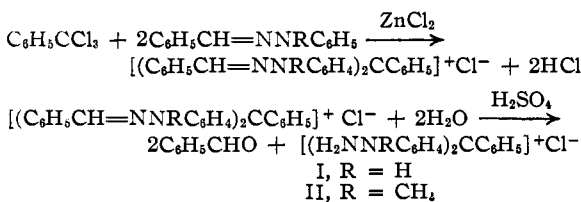


which like the A structure would produce a bathochromic effect. With molecules of this type a number of different resonance structures can be pictured which make significant contributions to the actual state of the molecule. Some of these structures can be associated with a bathochromic effect and other structures with a hypsochromic effect. The introduction of a substituent may stabilize several different structures which have opposing effects upon the color, and hence it is not easy to predict what the net result will be.

Lengthening the conjugated carbon chain in the terminal group, T<sub>1</sub>, has a bathochromic effect as can be seen by comparing the benzaldehyde dyes with the cinnamaldehyde dyes; also the crotonaldehyde compounds are deeper than the acetaldehyde dyes.

### Experimental

Dyes I and II were prepared by the reactions



The benzalhydrazone dyes were isolated as green lustrous crystals. The structure of these was not proven; however, evidence for the validity of the assigned structure is based upon the elementary analyses, the method of synthesis and the similarity of properties with the properties of other triphenylmethane dyes whose structures are well known. The dyes I, II and III were prepared by the hydrolysis of the corresponding benzalhydrazones. They were not isolated but were used in the solutions in which they were prepared.

**Benzalhydrazone of I.**—Benzotrichloride, benzalphenylhydrazone and anhydrous zinc chloride in molar ratio of 1:2:1 were intimately mixed and heated slowly to 80° with constant mixing by hand. The mixture turned green and was maintained at 80° for thirty minutes. After cooling to room temperature the product was broken up and washed with ligroin. It was then dissolved in methanol, precipitated with dilute hydrochloric acid, filtered and washed with dilute hydrochloric acid and air dried. The product was dissolved in chloroform, the chloroform solution washed with dilute hydrochloric acid and then water and dried with calcium chloride. The product was precipitated by the addition of ethyl ether, filtered and washed with ethyl ether. It was finally recrystallized from acetic acid and benzene. The pure product crystallized out after standing for several days. The analytical data suggest that the dye contains 1 mole of water.

*Anal.* Calcd. for C<sub>23</sub>H<sub>27</sub>N<sub>4</sub>ClH<sub>2</sub>: C, 74.5; H, 5.50; N, 10.5. Found: C, 74.0; H, 5.80; N, 10.3.

The benzalhydrazone of II was prepared in the same manner except that benzalphenylmethylhydrazone was used instead of benzalphenylhydrazone. For analysis this dye was converted to its methyl ether by dissolving the dye in methanol and adding sodium methylate solution until the green color disappeared. The methyl ether was precipitated with water, filtered and washed with water and dried over phosphoric anhydride.

The methyl ether can be converted back to the original colored dye by the addition of acid.

*Anal.* Calcd. for C<sub>26</sub>H<sub>32</sub>N<sub>4</sub>O: C, 80.5; H, 6.0; N, 10.4. Found: C, 79.6; H, 6.4; N, 10.4.

The benzalhydrazone of III was prepared in the same manner except that the benzotrichloride was replaced by the pseudo dichloride of *o*-sulfobenzoic acid.

*Anal.* Calcd. for C<sub>26</sub>H<sub>31</sub>N<sub>4</sub>SO<sub>3</sub>: C, 67.5; H, 5.04; N, 9.00; S, 5.15. Found: C, 67.1; H, 5.44; N, 8.43; S, 5.12.

To hydrolyze, 50 mg. of the dye was dissolved in 25 ml. of methanol, and 25 ml. of 20% by volume aqueous sulfuric acid was added. The solution was heated on a steam-bath for 0.5 to one hour or until the color of the solution has turned from green to red, the volume being kept approximately constant by the addition of 50% methanol. The benzaldehyde resulting from the hydrolysis evaporated off. After filtration the solution may be used as an aldehyde reagent. Its reactivity seems to decrease slowly and after two weeks it reacts sluggishly with aldehydes. We have found that the reagent performs satisfactorily for a period of a week after it has been prepared. For this reason it is advisable to store the dye as the hydrazone and to hydrolyze it as it is needed.

The qualitative tests with the various aldehydes were run by adding a drop of aldehyde to about 5 ml. of the reagent. The calibration curves for the quantitative determination of acetaldehyde and benzaldehyde, respectively, were obtained by measuring into 10-ml. volumetric flasks 3 ml. of the reagent Dye III and varying amounts from 0 to 0.8 ml. of aldehyde solution which contained 1 drop of aldehyde in 400 ml. of aldehyde-free methanol. The flasks were brought up to volume with methanol and were allowed to stand overnight. The readings were taken with a Beckman Spectrophotometer at the wave length where absorption of the dye formed with the particular aldehyde is at a maximum. This of course must be determined in advance using an excess of aldehyde. It was found that the concentration of dye in the reagent should be such that the contents of the flask which contains no aldehyde should have an optical density of 0.03 to 0.04 at the wave length at which the measurements will be taken.

### Summary

Three hydrazinotriphenylmethane dyes have been prepared and have been tested with various aldehydes to yield the corresponding hydrazones. An explanation has been provided for the color change accompanying this reaction which corrects misconceptions of previous workers. The absorption of these compounds in the visible spectral region has been measured. The usefulness of these dyes as reagents for the qualitative determination of aldehydes has been demonstrated and the possibility of using them for quantitative measurements has been indicated. The relationship between the color and constitution of these compounds has been discussed and principles set forth by previous workers on other dyes have been extended.

ABERDEEN, MARYLAND RECEIVED DECEMBER 30, 1948