The Color Reactions of Sympathomimetic Amines with Diazonium Compounds

By Karl H. Beyer

The coupling of p-nitrobenzenediazonium chloride with a sympathomimetic amine was first reported by Beyer and Skinner for the estimation of benzedrine (amphetamine).[1] The adaptation of this color reaction to biological media made possible an investigation of the inactivation and excretion of that compound by man and dogs.

The purpose of this research has been to study the relation of structure of the diazonium compound and of the sympathomimetic amine to the color formed when these agents are coupled. This knowledge is basic for the systematic identification as well as the colorimetric determination of these amines, as used in the example cited. However, it is believed that these experiments may be of interest to the investigator of diazo compounds and color formation as well.

Experimental

Preparation of the Diazonium Compounds.—Preparation from p-nitroaniline of the p-nitrobenzenediazonium chloride used in the test has already been described.[1]

o-Chloro-p-nitroaniline, sulfanilic acid, m-nitroaniline, and p-toluidine were also diazotized and used in a number of experiments. In general, 0.5 g. of the compound was mixed with 3 cc. of concentrated hydrochloric acid, heated almost to boiling and stirred for ten minutes. This was then diluted to 100 cc. with water and filtered.

Diazotization of o-chloro-p-nitroaniline hydrochloride was carried out at room temperature. To 5 cc. of filtered o-chloro-p-nitroaniline hydrochloride were added 1 cc. of hydrochloric acid and 3 cc. of cold 0.7% sodium nitrite. This was allowed to stand for eight minutes, then made up to 100 cc. with cold (approximately 0°) distilled water and placed in the refrigerator until used. Diazotization of the other solutions was carried out in an ice-bath. To 5 cc. of the hydrochloride in a 100-cc. volumetric flask was added 1 cc. of concentrated hydrochloric acid. This was allowed to stand for ten minutes in the bath and then 5 cc. of sodium nitrite solution was added. After six minutes distilled water was added up to the mark and the diazonium solutions allowed to stand in the ice-bath in a refrigerator for two hours before being used. These diazonium solutions were unstable at room temperature.

Coupling.—In this work equimolar concentrations of the organic ion were used (M/1600). To 1 cc. of the amine was added 5 cc. of the diazonium solution. This was allowed to stand for one hour at 21°. After an hour 5 cc. of 1.1% sodium carbonate was added slowly at such a rate that the drops could be counted. Fifteen minutes later

(1) Beyer and Skinner, J. Pharmacol. Exper. Therap., 68, 419 (1940).

1 cc. of 10% sodium hydroxide was added drop by drop and the solution allowed to stand for ten minutes for complete development of the color before diluting to 50 cc. with distilled water. This procedure for coupling applies to all the diazonium solutions and amines tested.

After diluting to about 50 cc., the aqueous colored solution was transferred to a 125-cc. separatory funnel and extracted with 15 cc. n-butanol. Following extraction of the color, the funnel and its contents were allowed to stand for fifteen minutes to permit separation of the two layers. The aqueous solution was drawn off and 10 cc. of the colored butanol layer transferred by means of a pipet to a test-tube. To the butanol was added 0.5 cc. of isopropanol. Shaking the tube cleared the colored solution completely. This final solution was used for the spectrophotometric analysis.

Interpretation of the Coupling Reactions

These reactions may conveniently be discussed under three headings: (1) coupling reactions and color formation of amines having no hydroxyl group on the ring, (2) coupling reactions of sympathomimetics having one hydroxyl group on the ring, and (3) reactions of compounds having 2 ortho hydroxyl groups on the ring.

Group 1. Primary amines having no hydroxyl group on the ring:

1 Benzylamine
2 β-Phenylethylamine
3 β-Phenyl-β-hydroxyethylamine
4 α-Isopropyl-β-phenylethylamine
5 β-Phenyl-n-propylamine
6 β-Phenylisopropylamine (amphetamine)
7 β-Phenyl-β-hydroxyisopropylamine (propadrine)
8 β-Phenyl-β-methylisopropylamine
9 β-(o-Methylphenyl)isopropylamine
10 γ-Phenylpropylamine
11 α-Phenyl-n-propylamine
12 α-Methyl-γ-phenylpropylamine

The following reactions may be suggested to account for the formation of color. Number 6 (amphetamine) may be taken as representative of the group.
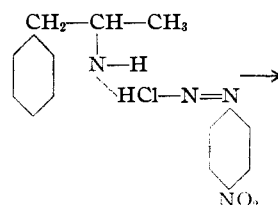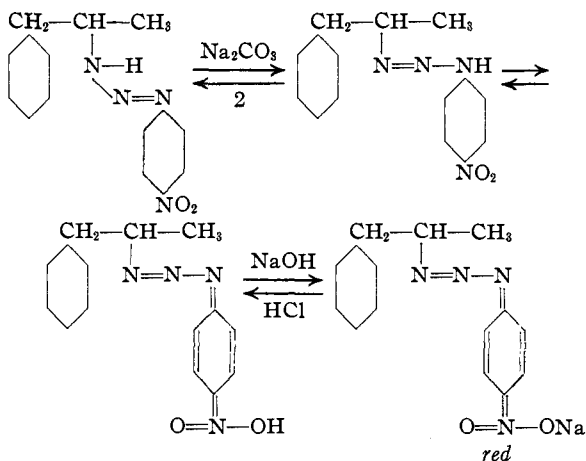
As evidence for this interpretation the following experiments are presented. 1. If sufficient sodium hydroxide to bring the pH quickly to approximate 11 is added at step 2, the red color does not appear. The reaction goes in another direction. 2. That migration of the hydrogen is necessary is evident by the fact that the secondary and tertiary amines of this group do not give a color.[2] 3. The nitro group must be present on the chromophore because if p-sulfonobenzenediazonium chloride is substituted for p-nitrobenzenediazonium chloride no color is formed on coupling. Moreover, p-methylbenzenediazonium chloride does not couple to produce a color. 4. Rearrangement of the chromophore from the benzenoid to the quinonoid form takes place in color formation for: (a) if a methyl is substituted for the nitro group, as in p-methylbenzenediazonium chloride, quinonization cannot take place and no color is formed. (b) If the nitro group is in the meta instead of the para position, as in m-nitrobenzenediazonium chloride, no color forms on coupling and addition of base. Here a quinonoid rearrangement cannot occur. (c) When o-chloro-p-nitrobenzenediazonium chloride is coupled with the amine, rearrangement of the molecule presumably does not take place since the color does not appear. 5. It is the sodium salt of the compound that is colored since the reaction can be reversed at will by shifting the pH back to 7.0 with dilute hydrochloric acid, in which case the red color fades to a pale yellow to reappear when the solution is made alkaline.

(2) None of these secondary or tertiary amines were found to produce a color when carried through the coupling process: (a) β-phenylethylmethylamine, (b) β-phenylisopropylmethylamine, (c) β-phenyl-β-hydroxyisopropylmethylamine, (d) β-phenyl-β-hydroxyisopropyldimethylamine, (e) β-phenylisopropyldimethylamine, (f) β-phenyl-β-hydroxyisopropyldiethylamine, (g) β-phenyl-β-hydroxy-2-piperidinopropane, (h) β-phenyl-β-hydroxy-2-morpholinopropane.
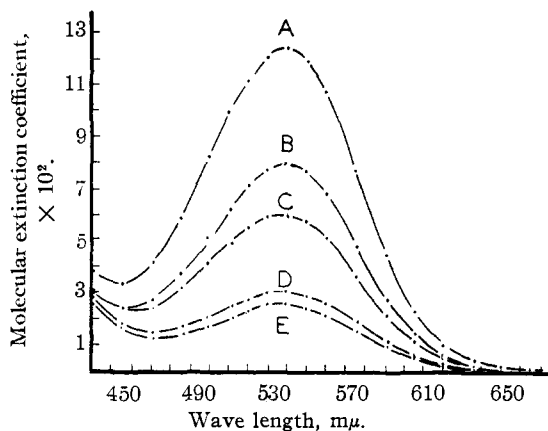
Fig. 1.—Representative spectrophotometric analyses of colors formed when coupling of the diazonium reagent is through the aliphatic primary amine. Curve A corresponds to β-phenylethylamine and α-isopropyl-β-phenylethylamine; B, benzedrine, o-methylphenylisopropylamine, β-phenylpropylamine, β-methyl-β-phenylisopropylamine; C, β-phenyl-β-hydroxyethylamine, γ-phenylpropylamine, α-methyl-γ-phenylpropylamine, α-phenyl-n-propylamine; D, propadrine; E, β-(4-hydroxyphenyl)-isopropylamine. Molecular extinction coefficient is expressed as $[K = E/Cl]$ where E is extinction coefficient, C is moles per liter or 6.25×10^{-5}, and l is length of path of light through the solution, 1 cm.

Figure 1 illustrates typical spectrum analyses of the butanol extracted colors resulting from the reaction of sympathomimetic amines of this group with p-nitrobenzenediazonium chloride. The instrument used was a Cenco Spectrophotelometer with a slit width permitting a 5 mμ spread of the spectrum. The length of the light path through the cell was 1 cm., and the wave length settings were at 5, 10 or 15 mμ intervals. These curves are representative of from 4 to 10 or more analyses of each compound.

Since several of these compounds gave nearly identical curves, a single curve has been used to represent sometimes two or three amines. Inspection of these curves will reveal that in the several instances their essential character is the same— indicating that these are related compounds.

The quantitative aspect of these curves is based on the assumption that coupling and the extraction of the colored compounds by butanol is complete. That this assumption is probably permissible is evidenced by our observation that the intensity of the color is a logarithmic function of the initial concentration of the amine, when that concentration is of the order represented in this work. In other words, if one plots the galvanometric values of intensity at the wave

length of maximal absorption for dilutions of the amines, from 0.1 to 0.01 mgm., against the concentration of the amine one obtains a curve the formula of which is $x = a - b \log y$, where $x =$ concentration of amine, a and b are constants and $y =$ galvanometric deflection.

Since these compounds are all of the same molar concentration, it may be concluded from the curves in Fig. 1 that light absorption is greatest when the aliphatic amino group is in the beta position with respect to the aromatic nucleus. The absorption maxima for all of this group that give a color are at a wave length of 525 ± 5 mμ.

Group 2.   Monohydric phenyl derivatives:

1   β-(4-Hydroxyphenyl)-ethylamine (tyramine)
2   β-(4-Hydroxyphenyl)-ethyl-methylamine
3   β-(4-Hydroxyphenyl)-β-hydroxyethylmethylamine (synephrine)
4   β-(3-Hydroxyphenyl)-β-hydroxyethylmethylamine (neosynephrine)
5   β-(4-Hydroxyphenyl)-isopropylamine (paredrine)
6   β-(4-Hydroxyphenyl)-isopropylmethylamine
7   β-(4-Hydroxyphenyl)-β-hydroxyisopropylmethylamine (suprifen)
8   β-(4-Hydroxyphenyl)-β-ketoisopropylmethylamine
9   β-(4-Hydroxyphenyl)-β-hydroxyisopropylamine (paredrinol)

The following reactions may be suggested to account for the formation of color. To illustrate these reactions we may take number 6, β-(4-hydroxyphenyl)-isopropylmethylamine.
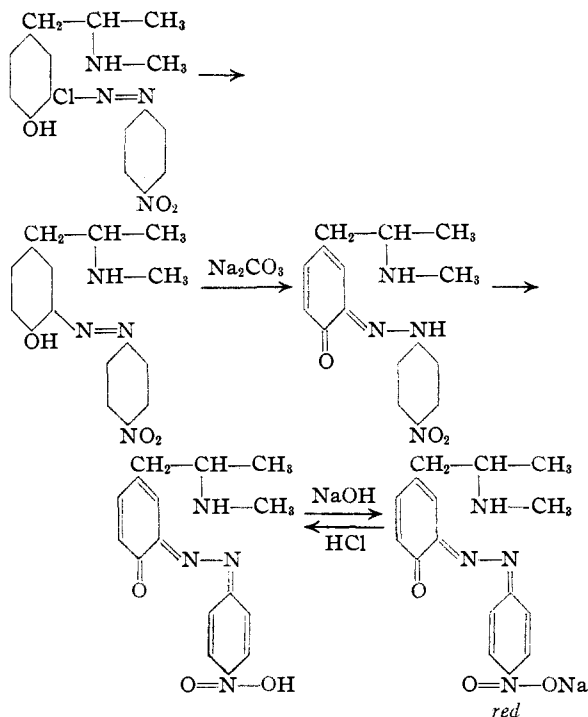
Evidence for these reactions is as follows: 1. In the example cited, coupling cannot take place through the aliphatic amino group for we have seen that a secondary amino group precludes the possibility of color formation from that source. 2. In reactions of diazonium compounds with phenols, the orientation of the chromophore is greatest for the carbon atoms para and ortho to the hydroxyl group of entrance. In this instance the ortho position is available. 3. While the o-quinonoid structure formed in the auxochrome may be responsible for the greater intensity of the color from the reactions of these compounds in general than the reaction of primary amines at this temperature, it alone cannot be held responsible for color production for: if o-chloro-p-nitrobenzenediazonium chloride is substituted for p-nitrobenzenediazonium chloride and the reaction carried out as usual, only a very faint color is produced instead of the red-violet color of much greater intensity with the latter diazonium reagent. 4. Coupled with m-nitrobenzenediazonium chloride which cannot undergo the quinonoid rearrangement the color formed is orange, suggesting that the nitro group is responsible for the shift in the absorption maxima to the longer wave lengths for: when phenol or β-(4-hydroxyphenyl)-isopropylmethylamine are coupled with p-methylbenzenediazonium chloride or p-sulfonobenzenediazonium chloride the color is yellow after the base is added. 5. Intensity is a function of concentration rather than substitution of more than one chromophore group into the ring of the parent amine for the relation can be expressed by the equation $x = a - b \log y$, an explanation of which has been given.

The presence of an aliphatic OH group in the alpha position with respect to the ring markedly influences the reactivity of the phenolic OH group. This we have shown to be true also for the enzymic inactivation of these compounds.[3] In general, those compounds which do not have the OH group on the side chain show a much more definite absorption band in the visible spectrum than when that group is present. An alpha keto radical has an inhibitory effect on the reaction of the aromatic OH group with the diazonium reagent similar to that of the corresponding aliphatic hydroxyl group, as is illustrated in Fig. 2. A secondary amine alone or together with the hydroxyl group on the side chain may similarly

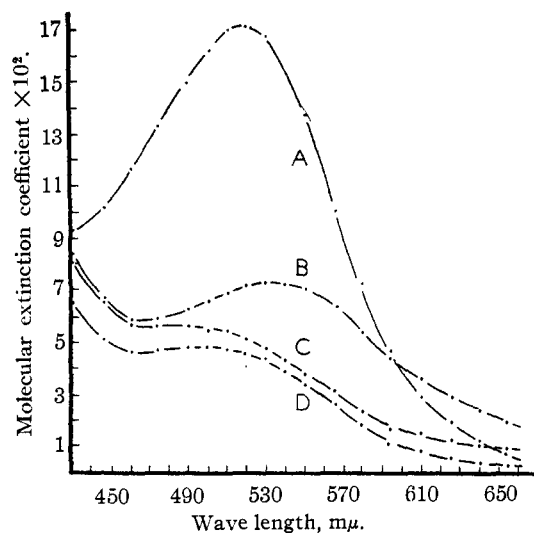(3) Beyer, J. Pharmacol. Exptl. Therapy, **71**, 151 (1941).

Fig. 2.—Absorption curves of Group 2 reactions with p-nitrobenzenediazonium chloride: A, neosynephrin; B, paredrine, β-(4-hydroxyphenyl)-ethylmethylamine; C, β-(4-hydroxyphenyl)-β-hydroxyisopropylmethylamine (suprifen); D, β-(4-hydroxyphenyl)-β-ketoisopropylmethylamine.

decrease or abolish any specific absorption band.

The inhibitory effect of the OH group in the side chain on the reactions of the aromatic OH radical is further brought out by the absorption curve for β-(4-hydroxyphenyl)-β-hydroxyisopropylamine, Fig. 1. Unlike the other compounds of this group which have the aliphatic OH group, this compound has a primary amino group. Ordinarily, where both an aromatic OH and a primary NH_2 coexist in the same molecule, the coupling affinity of the diazonium reagent is greater for the hydroxyl group. This is apparent when one contrasts the curves for tyramine, Fig. 3, and paredrine, Fig. 2, with those of compounds of Group 1. However, the absorption curve for β-(4-hydroxyphenyl)-β-hydroxyisopropylamine, Fig. 1, shows that the aliphatic OH group suppresses the affinity of the aromatic OH group for the diazonium compound below that of the primary amine; hence, coupling is in this case through the amino group. The absorption curve for this compound is identical in character to that of amines of the first group as contrasted with curves of compounds of Group 2.

Group 3. Catechol derivatives:

1   β-(3,4-Dihydroxyphenyl)-ethylmethylamine (epinine)
2   β-(3,4-Dihydroxyphenyl)-ethylamine
3   β-(3,4-Dihydroxyphenyl)-β-hydroxyethylamine (arterinol)
4   β-(3,4-Dihydroxyphenyl)-β-hydroxyethylmethylamine (adrenaline)

5   β-(3,4-Dihydroxyphenyl)-β-hydroxyisopropylmethylamine (cobefrin)
6   β-(3,4-Dihydroxyphenyl)-isopropylamine
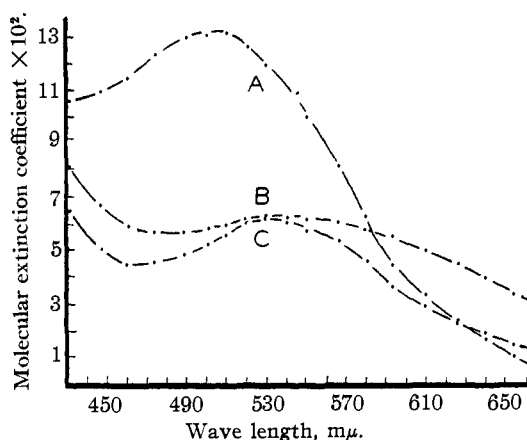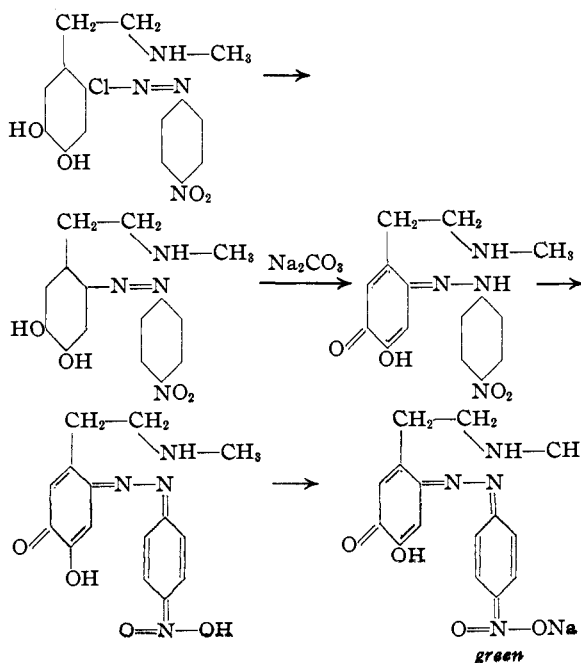7 β-(3,4-Dihydroxyphenyl)-β-hydroxyisopropyldimethylamine (methedrin)

Fig. 3.—Curves representative of Group 2 where coupling is through a single hydroxyl group on the ring: A, synephrin; B, β-(4-hydroxyphenyl)-isopropylmethylamine; C, tyramine. The concentration of these compounds is in every case 1 cc. of 6.25×10^{-5} molar solution with respect to the base, coupled at $21°$ and extracted with 15 cc. of n-butanol.

The test for this group with p-nitrobenzenediazonium chloride is characterized by the green color of the butanolic extract. The nature of the reactions in this group is not as certain because of the introduction of the second hydroxyl group into the ring. The following course is suggested for these reactions.
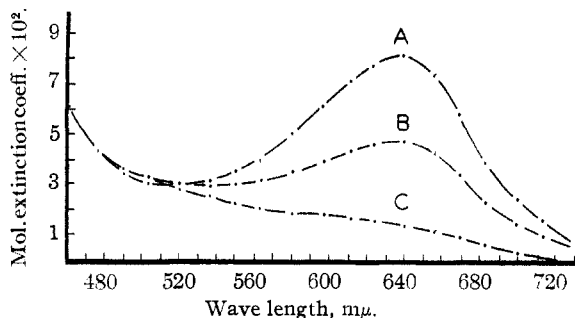
Fig. 4.—Analyses of the colors formed when sympathomimetic amines having a catechol nucleus (Group 3) are coupled with p-nitrobenzenediazonium reagent: A, epinine, cobefrin, and β-(3,4-dihydroxyphenyl)-isopropylamine; B, adrenalin, and β-(3,4-dihydroxyphenyl)-ethylamine; C, methedrin. Concentration and conditions are the same as for the other amines.

If one studies the reactions of similar compounds, omitting the side chain, some light can be thrown on the reactions. 1. Coupling p-nitrobenzenediazonium chloride solution with catechol or pyrogallol produces a yellow color in the acid medium becoming deeper yellow when the base is added. In neither of these is the color more soluble in butanol than in the alkaline aqueous medium. This indicates that the side chain and its substitutions are somehow partially responsible for the green color and the solubility of this color in butanol.

2. Bearing out the effect of the side chain on the characteristic of solubility are experiments on orcinol (1-methyl-3,5-dihydroxybenzene) and resorcinol (1,3-dihydroxybenzene), equimolar. Coupling these two compounds with diazotized p-nitroaniline produces in acid medium an orange color in the first instance and a yellow solution in the second. In basic solution resorcinol gives a much darker red color than does resorcinol. The color formed in the case of orcinol is very soluble in butanol while that from resorcinol is only very sparingly soluble in butanol. Presumably the

presence of the methyl C atom is responsible for this difference.

3. The presence of a primary or secondary amino group on the side chain is ultimately responsible for the green color, though the catechol nucleus is also requisite. This is suggested by the spectrophotometric analyses of these compounds, Fig. 4. In the case of primary and secondary amines of this group the green color is fairly distinct as reflected by the height of the absorption curve in the red end of the spectrum; that for methedrin (tertiary amine) is flattened and the color dark orange. In this case the characteristics of the amino nitrogen are suppressed by the complete substitution of hydrogen atoms by methyl groups.

From Fig. 4 it is apparent that some of these compounds give such similar spectra that they cannot be differentiated by this method. The color of compounds of this group other than methedrin have absorption maxima at a wave length of 640 ± 5 mμ.

Summary

The reaction characteristics of over thirty sympathomimetic amines with diazonium reagents have been described.

According to the spectrophotometric analyses of the colors formed as the result of these reactions, these compounds fall into three groups: Group 1, those having no hydroxyl groups on the benzene ring but having a primary aliphatic amino group; Group 2, where coupling is through a monohydric phenol nucleus; Group 3, those compounds having a catechol nucleus.