between specific rotations and atomic diameters previously found for the analogous derivatives of glucose, cellose, xylose and fructose. The methods of preparation, properties and analyses of fluoro-, chloro-, bromo- and iodo-triacetyl-*l*-arabinose are given.

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[CONTRIBUTION FROM THE DRUG CONTROL LABORATORY IN COÖPERATION WITH THE COLOR LABORATORY, BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

THE IDENTIFICATION OF PHENOLS BY MEANS OF THE SPECTROSCOPE[1]

BY SAMUEL PALKIN AND H. WALES

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In a previous paper[2] attention was called to the use of diazo-*p*-nitrobenzene as a test reagent for guaiacol sulfonate ("thiocol"). The advantages of this reagent over other diazonium salts for color reactions were shown.

The similarity in the behavior of the different phenols toward color-producing reagents and the limitation of the eye in distinguishing between the different shades produced, render color tests in themselves of little value in identifying the individual phenols. It is only when the colors produced are subjected to critical analysis by the spectroscope that positive identification becomes possible.

The frequency with which phenolic compounds occur in medicinal preparations, the small quantities in which they are found, and the admixture of various other compounds, make the problem of identifying any individual phenol in such products a very difficult one.

Gsell,[3] in a comprehensive review of the subject of phenol identification, has shown that wide differences in absorption spectra of the phthaleins of the various phenols exist, and that these afford a very positive means for their identification. Once prepared, the phthaleins are no doubt of diagnostic value, but the exacting conditions (such as absence of water, limitation in temperature range and comparative freedom from contaminating substances) necessary for the phthalein condensation detract much from the usefulness of this reaction, particularly as applied to complex medicinal preparations.

Investigation by the authors has shown that the ease with which the azo dyes can be prepared, under the conditions most common in practical analysis, makes this means particularly well adapted for the identification of phenols in medicinal preparations. The most useful general reagent

[1] Presented at the Washington meeting of the American Chemical Society, April, 1924.

[2] S. Palkin, *J. Ind. Eng. Chem.*, **10**, 618 (1918).

[3] Gsell, *Z. anal. Chem.*, **55**, 417 (1916).

for this purpose was found to be diazo-*p*-nitrobenzene. When properly prepared this reagent has been kept in a refrigerator for months without decomposition. By the method described below less than 0.01 mg. of the phenols examined has been detected with this reagent. As little as 0.5 mg. was sufficient for positive identification under ordinary conditions and this quantity sufficed for the spectroscopic examination in three different solvents. The dyes thus prepared show marked differences in absorption spectra in different solvents.

Experimental Part

Preparation of the Azo Dyes

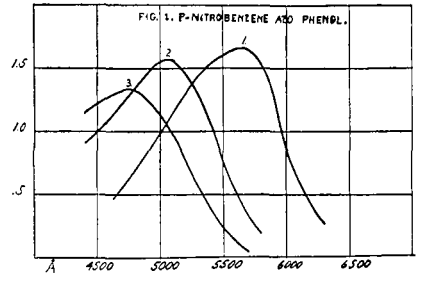
The reaction involved in the preparation of these dyes is the usual one for the formation of azo dyes which may be expressed: $RNO_2-N=N-Cl + R'OH \longrightarrow RNO_2N-N-R'OH + HCl$.

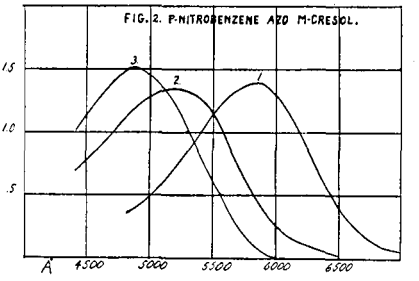
(a) **The Reagent (Diazo-*p*-nitrobenzene).**—A solution of 0.14 g. of pure *p*-nitro-aniline in 8 cc. of water containing 2 cc. of conc. hydrochloric acid (prepared by warming on the steam-bath) was cooled, a piece of ice was added, and a cooled solution of sodium nitrite (0.075 g. in 3 cc. of water) was added slowly with stirring, while the temperature was kept between 5° and 8°. The diazo solution was kept in a refrigerator.

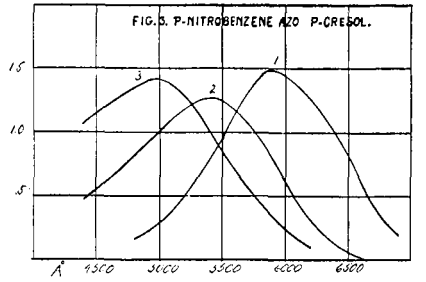
(b) **The Dye.**—To the cool, slightly alkaline solution of the phenol (in the presence of ice) a quantity of the diazo-*p*-nitrobenzene corresponding to less than a molecular equivalent of this reagent was added. (1) The dye thus formed was salted out, filtered and washed, or (2) the solution was made acid and extracted with chloroform, washed several times with water, and the chloroform extract evaporated to dryness. In the presence of sulfonated phenols, chloroform does not extract the dye or does so to only a very slight extent. The extraction in such cases was made with ethyl ether instead of chloroform. Portions of this residue were taken up in the various solvents, water, alcohol, acetone, with the addition of alkali, and spectroscopic observations were made as described below. The absorption curves shown in the paper serve as reference standards for the identification of unknown samples.

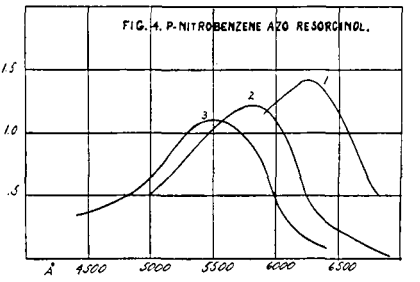
(c) Examinations of "unknowns" were made for the purpose of testing the efficacy and accuracy of the method of identification. The operator making the spectroscopic measurements did not know what kind of phenol was used. The experiments were carried out as follows. A piece of ice and a drop of the reagent were added to a small quantity of the solution containing the phenol. The solution was then made alkaline, whereupon the dye was produced. Subsequently a drop or two of the reagent and a little more alkali were added. The extent of the phenol present was thus indicated by the increase or non-increase in intensity of color. (It is best to carry out the test with less than the equivalent quantity of reagent,

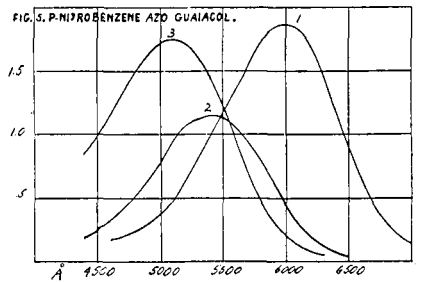
as it is preferable, though not essential, to have the phenol in excess of the diazonium salt.)

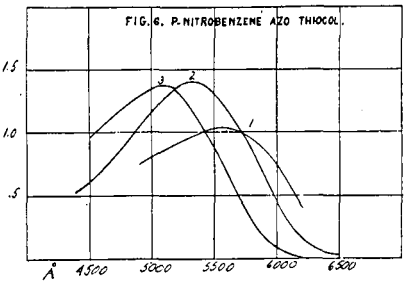


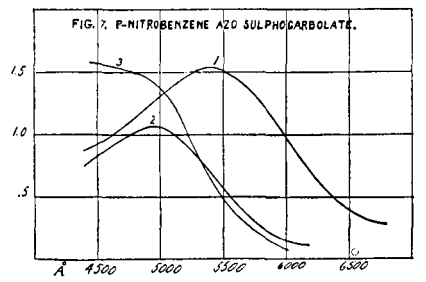


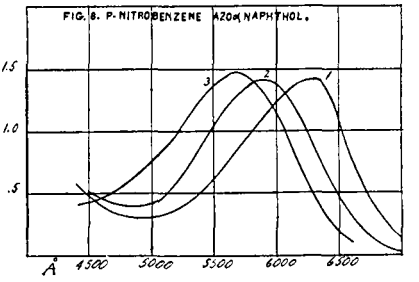






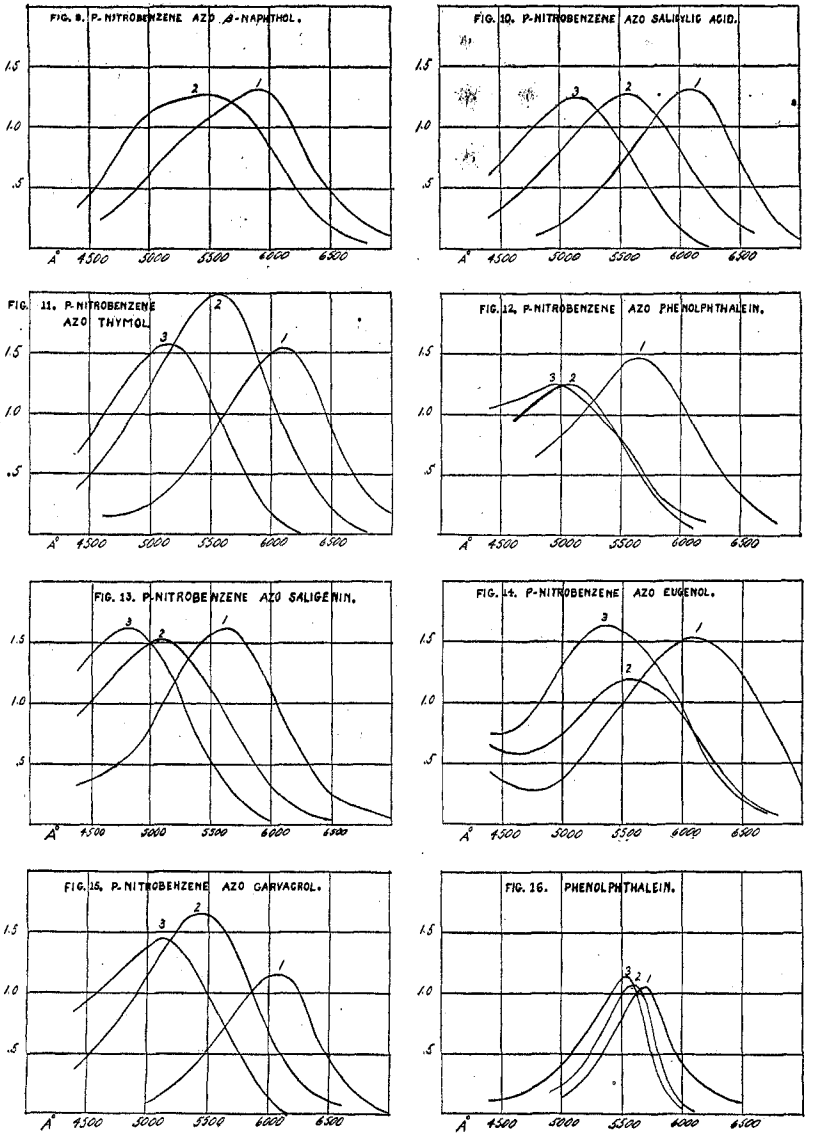






The solution containing the dye was then acidified with hydrochloric acid and extracted in a separatory funnel with chloroform as described under (**b**) (2).

A blank test on the diazo reagent is always desirable, to insure against errors due to the presence of interfering by-products.



Spectroscopic Examination

The residue from the chloroform extract was dissolved in a few cubic centimeters of alcohol, the solution divided into three portions in small beakers, and evaporated to dryness. These three portions were dis-

solved, respectively, in alkaline (1) acetone, (2) alcohol and (3) water. These solvents were made by adding one drop of alcoholic potassium hydroxide[4] to 20 cc. of acetone or 95% alcohol or in the aqueous solutions by adding a slight excess of potassium hydroxide over that required to bring out the color. On account of the shift in the maximum on changing from alcohol to acetone as a solvent, it is apparent that the quantity of alcohol in the acetone must be kept at a minimum. For this reason only one drop of alcoholic potassium hydroxide should be used. The concentration of alkali in the alcoholic solutions exerts a slight influence on the position of the absorption maximum and so must be kept fairly uniform. These difficulties disappear in the aqueous solutions and no precautions beyond having just an excess of alkali are necessary.

All spectroscopic determinations were made with a Hilger spectrometer equipped with a Nutting photometer. A 1cm. cell was used throughout and each solution was diluted with its particular solvent until an extinction coefficient of approximately 1.5 was shown at the absorption maximum. This gave nearly identical concentrations in all cases.

The acetone solutions in several cases are bleached fairly rapidly by the carbon dioxide in the air and if possible the determinations should be made in a stoppered cell.

TABLE I

COLOR OF SOLUTION OF THE *p*-NITROBENZENE AZO DYES FROM THE VARIOUS PHENOLS IN DIFFERENT SOLVENTS

		Acetone	Alcohol	Water
I	Phenol	Purplish-blue	Red (brownish)	Brownish-yellow
II	*m*-Cresol	Blue	Purplish-red	Red-brown
III	*p*-Cresol	Blue	Purple	Orange
IV	Resorcinol	Blue	Purple	Purple
V	Guaiacol	Blue	Purple	Red
VI	Thiocol	Blue	Purple	Red
VII	Sulfocarbolate	Blue	...	Red-brown
VIII	α-Naphthol	Dark blue	Blue	Purplish-blue
IX	β-Naphthol	Blue	Purple	Not soluble
X	Salicylate	Blue	Purple	Wine red
XI	Thymol	Blue	Purple	Red
XII	Phenolphthalein	Part ppt.	Part ppt.	Red-brown
XIII	Saligenin	Blue	Purple	Red
XIV	Eugenol	Blue	Purple	Red
XV	Carvacrol	Blue	Purple	Red

Figs. 1 to 16 show the absorption curves of the various dyes in the solvents: 1, acetone; 2, alcohol; and 3, water.

[4] The alcoholic potassium hydroxide, was a solution of about 20 g. in 100 g. of absolute ethyl alcohol or, preferably, methyl alcohol.

TABLE II

ABSORPTION-SPECTRUM MAXIMA OF AZO DYES FROM PHENOLIC COMPOUNDS

		Acetone Å.	Alcohol Å.	Water Å.
I	Phenol	5675	5050	4750
II	*m*-Cresol	5850	5200	4900−
III	*p*-Cresol	5900	5400	5000−
IV	Resorcinol[a]	6250	5800	5500
V	Guaiacol	6000	5400	5100
VI	Thiocol	5500	5350	5100
VII	Sulfocarbolate	5400	5000	..
VIII	α-Naphthol	6300	5900	5700−
IX	β-Naphthol	5875	5500[b]	..
X	Salicylate	6100−	5550	5150
XI	Thymol	6100	5600−	5150
XII	Phenolphthalein[c]	5700−	5100−	4950
XIII	Saligenin	5650	5100−	4800+
XIV	Eugenol	6100−	5550	5300
XV	Carvacrol	6050	5450	5100−

[a] A large excess of alkali was necessary in IV—about 1 cc. in alcohol.

[b] The curve is very flat; the value is an approximation.

[c] Phenolphthalein itself (Fig. 16) showed the following maxima: in acetone 5525 Å.; in alcohol, 5600 Å.; in water, 5700 Å.

TABLE III

ABSORPTION-SPECTRUM-MAXIMA OF UNKNOWN DYES AND THOSE OF COMPARISON SUBSTANCES

No.	Acetone Å.	Alcohol Å.	Water Å.
1 Unknown	5675	5050	4750
Phenol	5675	5050	4750
2 Unknown	6000	5400	5100
Guaiacol	6000−	5400	5100
3 Unknown	6300	5900	5700+
α-Naphthol	6300+	5900	5700
4 Unknown	6100	5600	5150
Thymol	6100+	5600	5150

Summary

The diagnostic value of absorption spectrum examination as a supplement to color tests is pointed out.

The absorption spectra of azo dyes prepared from certain phenols, by coupling with azo-*p*-nitrobenzene, have been examined in three solvents and the data comprising absorption curves and tables of absorption-spectrum maxima are recorded for reference.

The application of this procedure to the detection and the indentification of small amounts of phenols is discussed and a method adapted particularly to the examination of medicinal preparations is outlined.

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