[CONTRIBUTION FROM THE DEPARTMENTS OF PHARMACOLOGY AND TROPICAL MEDICINE, HARVARD MEDICAL SCHOOL]

PARA-ARSONOBENZENE-AZO-PHTHALEINS

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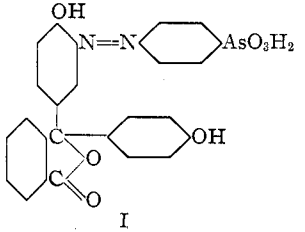
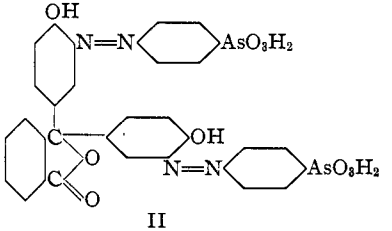
p-Arsonobenzene-azo-phthaleins may be prepared readily by adding diazotized arsanilic acid to an alkaline solution of a phthalein; derivatives obtained from phenolphthalein, phenoltetrachlorophthalein, fluorescein, dibromofluorescein and phenolsulfonephthalein are reported herein. When equivalent quantities of arsanilic acid and the phthalein are employed, the yield is practically quantitative, and the arsenic content of the reaction product is that calculated for the particular arsonobenzene-azo-phthalein. Thus, in the case of phenolphthalein, the material contains 13.5% of arsenic as compared with a calculated value of 13.7%. The material isolated from the reaction mixture is, however, a mixture of the unchanged phthalein and its mono- and di-arsonobenzene-azo derivatives. The phenolphthalein product referred to above can be fractionated into phenolphthalein, mono-arsonobenzene-azo-phenolphthalein and di-arsonobenzene-azo-phenolphthalein. The separation of these substances is accomplished by fractional precipitation from aqueous alkaline solution with alcohol. The sodium salt of the disubstituted phthalein separates when alcohol is added to a comparatively dilute solution of the mixture in aqueous sodium hydroxide; the sodium salt of the mono-substitution product separates only when alcohol is added to the concentrated mother liquor from the first fraction, and the sodium salt of the unchanged phthalein remains in the alcoholic solution.

Although definite proof has not been obtained regarding the position in the phthalein molecule where coupling occurs, it is practically certain that reaction takes place *ortho* to the phenolic hydroxyl group.[1] This conclusion is based on the known behavior of phthaleins in substitution reactions and the behavior of diazonium compounds with *para* substituted phenols. The phenolphthalein derivatives are, therefore, represented by Formulas I and II.



I



II

[1] White [THIS JOURNAL, **42**, 2357 (1920)] did not demonstrate rigidly the position occupied by the mercury in his mercury phthaleins but concluded that mercuration occurred *ortho* to the hydroxyl group.

This study was undertaken primarily to determine the trypanocidal activity of arsenical compounds of this type. The derivatives of phenolphthalein and phenoltetrachlorophthalein are more toxic than those of fluorescein and dibromofluorescein; the sulfonephthalein compound has an extremely low toxicity. None of these substances gives more than slight indications of trypanocidal activity when tested in vivo against *Tr. brucei*. Excepting in the cases of the chlorophthalein derivatives, intravenous injection of the intensely colored alkaline solutions of these azophthaleins causes albino rats to become highly colored immediately; the hair alone remains unchanged. Excretion of the material sets in rapidly; in some cases the urine is distinctly colored immediately after the injection has been completed. The rate of excretion, as determined by the arsenic content of the excreta,[2] varies greatly. Thus, in six hours the di-arsono-azo derivatives of phenolphthalein and fluorescein are excreted to the extent of 48 and 80%, respectively. In each case, the urine is very similar to the original solution with respect to color and tinctorial power.

Sen and Sett[3] studied "the effect of the addition of azo groups to fluoresceins" and concluded that "by the introduction of an azo group, fluorescence is more or less diminished and the shade is deepened to a certain extent." Only one compound, *o*-4-sulfobenzene-azo-fluorescein, was examined. All of the substances prepared in the present study are of the same general type as this sulfo compound, and one of them is the arsenic analog of the above-mentioned sulfo compound. Colorimetric observations on the eight arsenicals reported herein are in general agreement with the above quotation. The introduction of one and two *p*-arsonobenzene-azo groups into a colorless phthalein produces orange and brown colors, respectively, in the solid substances. In alkaline solution the mono-substituted derivative is slightly more deeply colored and the disubstituted derivative is much more deeply colored than the original phthalein. In the case of the fluoresceins, one arsonobenzene-azo group has very little influence on the color of the material in the solid state or in alkaline solution; a second substituent, however, causes a marked deepening of the color of both the solid and dissolved material. None of the new compounds gives any indications of usefulness as an indicator.

Experimental Part

As the method of preparation is practically the same in each case, one example will be given in detail. Arsanilic acid (4.3 g.) is dissolved in 43 cc. of water and 4.1 cc. of hydrochloric acid (d., 1.19) and diazotized between 0° and 6° with a solution of 1.38 g. of sodium nitrite in 8.6 cc. of water during mechanical stirring. After 15 minutes the diazo solution is

[2] Unpublished results of Dr. Anne S. Minot.

[3] Sen and Sett, THIS JOURNAL, **46**, 113 (1924).

siphoned into a solution of 6.5 g. of fluorescein in 55 cc. of water containing 4.5 g. of sodium hydroxide which is in an ice-bath and being stirred mechanically. At the end of 30 minutes the reaction mixture is acidified with 10 cc. of concd. hydrochloric acid, and the resulting pasty mass is left in the ice-bath for 30 minutes and then centrifuged. The gelatinous precipitate is washed thrice with water and centrifuged each time, and dried in an oven at 80°. The reaction product is an amorphous, red powder which is a mixture.

To fractionate this material it is suspended in 30 cc. of warm water and treated with 4 cc. of 12.5 *N* sodium hydroxide solution; the system is warmed until the solid has dissolved and is then centrifuged. The deep red solution is decanted from a small quantity of slimy material into 120 cc. of alcohol; a deep red oil separates. The material is placed in the icebox for an hour and then centrifuged. The clear, supernatant alcohol is decanted and the centrifuge tube is rinsed with alcohol. A solution of the residual oil in 50 cc. of water is acidified with 4 cc. of concd. hydrochloric acid, and the resulting brown precipitate is separated by centrifuging, washed thrice with water and dried in an oven at 80°. The brick-red, amorphous powder (3.5 g.) is slightly impure *o,o'*-di-4-arsonobenzene-azo-fluorescein.

The alcoholic mother liquor and rinsings from the oil are evaporated to about 15 cc. and poured into 35 cc. of alcohol; a dark colored oil separates. The material is left at room temperature overnight; the supernatant alcohol is decanted, and the tube is rinsed with alcohol. The oil is dissolved in water and acidified as in the previous case. The deep orange, amorphous powder (2.2 g.) is slightly impure *o*-4-arsonobenzene-azo-fluorescein.

The oil obtained by evaporating the alcoholic mother liquor and rinsings from the second oil to 5 cc. and precipitating with 35 cc. of alcohol gives pure *o*-4-arsonobenzene-azo-fluorescein; yield, 0.9 g.

When the alcoholic liquor from the third oil is evaporated to a sirup and the latter is dissolved in 30 cc. of water, acidification yields 2.2 g. of an orange powder which is a mixture of fluorescein and some *o*-4-arsonobenzene-azo-fluorescein.

The disubstituted fluorescein may be obtained in a pure condition by redissolving it in aqueous alkali, precipitating with alcohol and acidifying the oil. Also, the yield of monosubstituted material may be increased by dissolving the final fraction in the smallest possible quantity of aqueous alkali, precipitating with a large quantity of alcohol and acidifying an aqueous solution of the resulting oil. In the work reported here no attempt has been made to fractionate the products completely; the prime object was to secure sufficient material for pharmacological studies. The reaction products obtained from the various phthaleins are reported in Table I; the yields are given in Table II.

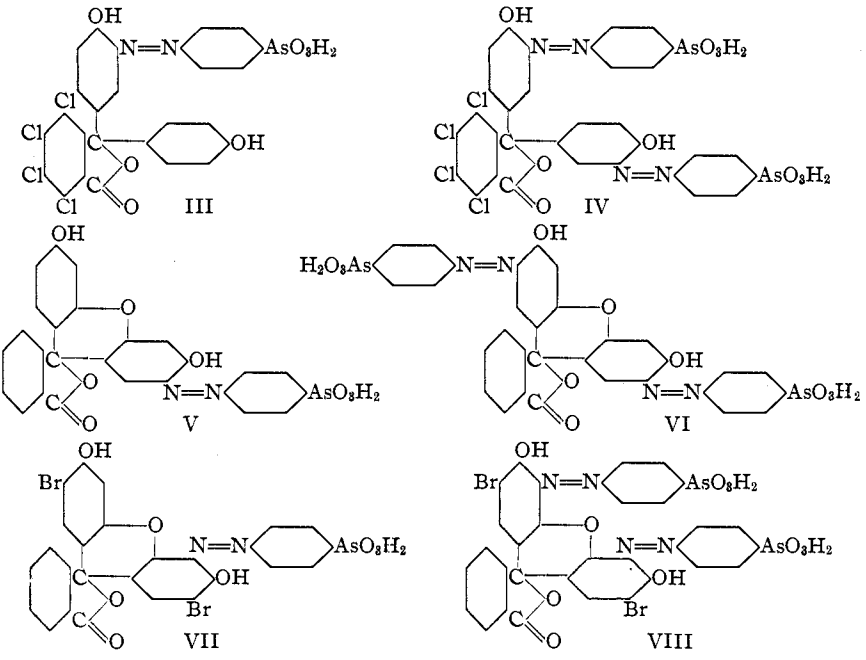


TABLE I

ARSONOBENZENE-AZO-PHTHALEINS

Formula	Substance	Empirical formula	% As Found[a]	% As Calcd.	Total dose[b] Mg./kg.
I.	*o*-4-A[c]-azo-phenolphthalein	$C_{26}H_{19}O_7N_2As$	14.1	13.7	< 100
II.	*o,o'*-Di-4-A-azo-phenolphthalein	$C_{32}H_{24}O_{10}N_4As_2$	19.3	19.4	180
III.	*o*-4-A-azo-phenoltetrachlorophthalein	$C_{26}H_{15}O_7N_2Cl_4As$	9.9	11.0	< 300
IV.	*o,o'*-Di-4-A-azo-phenoltetrachloro-phthalein	$C_{32}H_{20}O_{10}N_4Cl_4As_2$	16.2	16.4	< 500
V.	*o*-4-A-azo-fluorescein	$C_{26}H_{17}O_8N_2As$	13.5	13.4	> 260
VI.	*o,o'*-Di-4-A-azo-fluorescein (crude)	$C_{32}H_{22}O_{11}N_4As_2$	21.0	19.1	> 280
VII.	*o*-4-A-azo-dibromofluorescein	$C_{26}H_{15}O_8N_2Br_2As$	10.6	10.5	>1000
VIII.	*o,o'*-Di-4-A-azo-dibromofluorescein	$C_{32}H_{20}O_{11}N_4Br_2As_2$	15.6	15.8	..

[a] Arsenic determinations were made according to Ewins' method.

[b] Determined by intravenous injection of alkaline solutions into albino rats.

[c] A = arsonobenzene.

TABLE II

YIELDS OF PHTHALEINS

Starting material	Amount	Reaction products
Phenolphthalein	7.3 g.	4 g. of I[a]; 0.8 g. of II; 3.2 g. of I and II in about equal amounts.
Phenoltetrachlorophthalein	3 g.	0.8 g. of III; 1 g. of IV; 1.5 g. of original phthalein and III.
Fluorescein	6.5 g.	3.1 g. of V; 3.5 g. of VI; 2.2 g. of fluorescein and V.
Dibromofluorescein	8.1 g.	3.5 g. of VII; 0.4 g. of VIII; 3.5 g. of dibromofluorescein and a small amount of VII, unidentified (2 g.).

[a] These numbers refer to the compounds as listed in Table I.

These arsonobenzene-azo-phthaleins are colored, amorphous powders which are insoluble in water, hydrochloric acid, ether, chloroform, benzene and methyl acetate but soluble in alcohol, acetone, glacial acetic acid, concd. sulfuric acid and aqueous alkalies.

In the case of phenolsulfonephthalein the experimental procedure had to be varied slightly. After diazotizing 1.22 g. of arsanilic acid, coupling with 2 g. of phenolsulfonephthalein and acidifying as indicated above, the reaction mixture is saturated with salt and left in the icebox overnight. The red precipitate is removed by centrifuging, washed twice with saturated salt solution and dried at 83°. The crude product is extracted with five 100cc. portions of warm absolute alcohol, and the clarified extract is evaporated to dryness. The dark red powder (2.85 g.) is readily soluble in water, methyl and ethyl alcohols, fairly soluble in acetone

TABLE III

COLORS OF PHTHALEINS

	Substance	Alkaline solution, 10 mg. in 10 cc. of 0.04 *N* NaOH: Color	Fluorescence
1.	Phenolphthalein	Cerise	.....
2.	Mono-azo deriv.	Red	.....
3.	Di-azo deriv.	Deep red	.....
4.	Phenoltetrachlorophthalein	Deep red, violet tinge	.....
5.	Mono-azo deriv.	Deep reddish-purple	.....
6.	Di-azo deriv.	Very deep bluish-purple	.....
7.	Fluorescein	Orange-yellow	Green
8.	Mono-azo deriv.	Orange-yellow	Greenish-yellow
9.	Di-azo deriv.	Red	Olive-green
10.	Dibromofluorescein	Orange	Grass-green
11.	Mono-azo deriv.	Orange	Yellowish-green
12.	Di-azo deriv.	Deep orange	Greenish-yellow
13.	Phenolsulfonephthalein	Red	.....
14.	Azo deriv.	Very deep red	.....

	Color of solid	Sulfuric acid solution, 10 mg. in 10 cc. of concd. acid: Color	Fluorescence
1.	Colorless	Deep orange	.....
2.	Orange	Deep orange	.....
3.	Dark brown	Deep red	.....
4.	Colorless	Deep pink	.....
5.	Brown-orange	Reddish-orange	.....
6.	Dark brown	Deep red	.....
7.	Red-orange	Yellow	Weak green
8.	Dark orange	Yellow	Green
9.	Brick red	Deep red	Dull green
10.	Deep orange	Yellow	Very weak green
11.	Red-orange	Yellow	Very weak green
12.	Deep red	Deep orange	Very weak green
13.	Deep red	Deep orange	.....
14.	Very deep red	Deep red	.....

and glacial acetic acid but insoluble in ether. Although this material is probably a mixture, it was not fractionated on account of the complications arising from the ease with which it is soluble in water. The material contains 11.2% of arsenic and 4.2% of sulfur and is tolerated in doses above 1.8 g./kg.

Table III contains some observations regarding the color of these compounds; for the purpose of comparison the phthaleins used in preparing these arsenicals are included.

The expenses necessary for the pursuance of this investigation have been met in part from a fund for research in the Department of Tropical Medicine, donated by a citizen of Boston.

Summary

1. When a solution of *p*-arsonobenzene diazonium chloride is added to an alkaline solution of an equivalent quantity of a phthalein, coupling takes place, but the reaction product is a mixture of unchanged phthalein and its mono- and di-arsonobenzene-azo derivatives.

2. The mixture can be resolved by fractional precipitation from aqueous alkaline solution with alcohol.

3. *p*-Arsonobenzene-azo derivatives have been prepared from phenolphthalein, phenoltetrachlorophthalein, fluorescein, dibromo-fluorescein, and phenolsulfonephthalein.

4. The derivatives of phenolphthalein and phenoltetrachlorophthalein are more toxic than those of the other substances.

5. None of these arsenical compounds is trypanocidally active.

6. The introduction of *p*-arsonobenzene-azo groups deepens the shade but does not alter the character of the color.

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BUTYRIN

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Experimental work in the preparation of butyrin led the authors to believe that the statements as to the properties of butyrin given in Beilstein and in other chemical tables, especially that giving the boiling point of tributyrin as 285°, are in error. It was believed that in the preparation of the butyrin from which these constants were determined, not sufficient care had been taken to eliminate the lower glycerides. Investigation was undertaken, therefore, for the purpose of preparing and isolating the pure triglyceride, and establishing its properties more accurately.

Butyrin was prepared by the direct esterification of glycerol and butyric