1-phenylethyl-3-carbethoxy-4-piperidyl-*p*-aminobenzoates has been included.

3. This pharmacological report indicates that the cyclohexyl group does not correspond to the higher alkyl groups in its physiological effect and is a much less desirable N-substituent group than the phenylethyl group for this particular type of local anesthetic.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE JOHNS HOPKINS UNIVERSITY]

AZO DYES CONTAINING ANTIMONY. II

BY FITZGERALD DUNNING AND E. EMMET REID

RECEIVED JULY 21, 1927 PUBLISHED NOVEMBER 5, 1927

In a previous paper,[1] the preparation of a series of azo dyes containing antimony has been described. These compounds were prepared for a study of their possible therapeutic action. Since that time another series of dyes and several miscellaneous antimonials have been prepared. These compounds have been tested as to their activity against trypanosomes and as to their toxicities. The pharmacological findings, which are summarized here, will be published more fully elsewhere.

As these compounds are, for the most part, dyes, it was thought of interest to ascertain the effect of the antimony group on the color. Dyes containing the group $-SbO_3H_2$ have been contrasted with those having the $-SO_3H$ group and with some containing neither.

Results

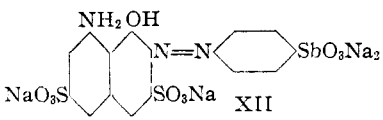
The compounds which have been prepared are for the most part derivatives of stibanilic acid, *p*-aminophenylstibinic acid—$H_2NC_6H_4SbO_3H_2$. The method of preparation of this compound has been further improved. This consists in breaking down the molecular compound formed by diazotized *p*-amino-acetanilide and antimony trichloride with hot sodium hydroxide. Thus stibanilic acid is obtained from the molecular compound in one step.

The first series of compounds, Nos. I to VI, described in the previous paper, were obtained by coupling diazotized stibanilic acids with substituted phenols. In the second series, diazotized stibanilic acid has been coupled with dimethyl- and diethylaniline to form *p*-dimethylamino-azobenzene-4′-stibinic acid and the corresponding diethyl compound (Nos. VII and VIII). The disodium salts, $Na_2O_3SbC_6H_4N{:}NC_6H_4N(R)_2$, are normally formed and are readily soluble in water. The solutions are comparatively stable.

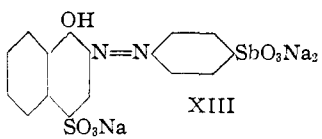
The third series has been prepared by coupling diazotized stibanilic acid with various substituted naphthalene sulfonic acids.

[1] Dunning and Reid, THIS JOURNAL, **48**, 2959 (1926).

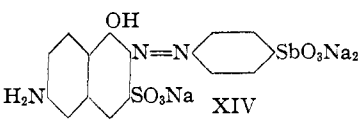
The intermediates used were as follows: 1,8-naphthylamine-3,6-disulfonic acid (H-acid); 1-naphthol-4-sulfonic acid (Neville and Winther's acid); 5,2-naphthylamine-7-sulfonic acid (J-acid); 2-naphthol-3,6-disulfonic acid (R-salt); 2-naphthylamine-3,6-disulfonic (amino R salt); 1,8-dinaphthol-3,6-disulfonic acid (chromotrope), to form compounds XII, XIII, XIV, XV, XVI and XVII, respectively. The structural formulas are



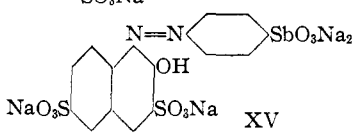
XII



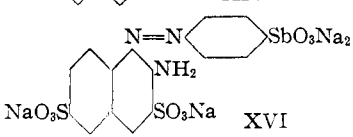
XIII



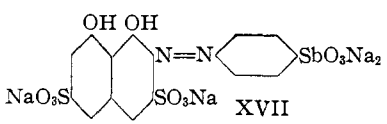
XIV



XV



XVI



XVII

Methylanthranilate, *p*-aminodimethylaniline and benzidine have also been diazotized, coupled with antimony trichloride and treated with sodium hydroxide to give the following compounds: methyl *o*-stibono-benzoate, *p*-dimethylaminophenylstibinic acid and *p,p'*-dibenzenestibinic acid.

The sodium salts of all the compounds described have been tested as to their effectiveness against trypanosomiasis. For this purpose the white (albino) rat infected with *Trypanosoma equiperdum* has been used. All of the compounds of the first and second series have proved to be totally ineffective. With the third series, however, positive results have been obtained. Compound XII (as indicated above) has proved to be the most effective and compounds XVI and XIV follow in order.

The compounds which have been found to be effective possess approximately the same structural formulas, as the pharmacological table of findings will show. The presence of one amino group and one sulfonic acid group, and preferably two sulfonic acid groups, in the naphthalene intermediate, seems to be necessary, as an inspection of the formulas of compounds XII, XIV and XVI will show. These findings are in keeping with the conclusions of Ehrlich[2] and of Nicolle and Mesnil[3] working with benzidine dyes.

That antimony is necessary is witnessed by the fact that analogous dyes made from sulfanilic acid have no trypanocidal action.

[2] Ehrlich, *Berl. klin. Wochschr.*, **44**, 233, 280, 310, 341 (1907).

[3] Nicolle and Mesnil, *Ann. inst. Pasteur*, **20**, 417, 513 (1906).

Experimental

In following any of the general methods[4,5] for the preparation of stibanilic acid, several difficulties are encountered.

Not only is the material hard to handle mechanically, but the yields are generally low. In the first place, on treating the diazotized amine solution with antimony trichloride, the oxychloride of antimony precipitates out unless the solution is strongly acid. When a strong sodium hydroxide solution of antimony oxide is used, the oxide precipitates. The greatest difficulty, however, is caused by the production of a stiff foam which prevents thorough mixing of the reactants and causes much trouble mechanically. This foam subsides only after standing for a considerable length of time (24 hours) and occasions much decomposition. To overcome this, we experimented with various foam-breakers such as ether, alcohol, acetone, etc., but these all seemed to cause decomposition and were discarded. We have developed, however, two methods which have proved fairly successful. One, a modification of the method of Schmidt, was reported previously. The other is shorter, in that two steps are combined.

A mixture of *p*-amino-acetanilide (150 g.), recrystallized after boiling with charcoal, and antimony trioxide (145 g.) in one liter of water containing sufficient 33% hydrochloric acid to dissolve the trioxide (600 cc.) was warmed until everything dissolved. On cooling, white crystals of the hydrochloride of the amine separated out. This mixture was cooled to 0° and diazotized with a solution of 69 g. of sodium nitrite in 460 cc. of water. The nitrite solution was introduced through a capillary at the bottom of the beaker, with mechanical stirring. During this slow addition a yellow, crystalline (rectangular plates) compound formed. This is the double salt of the type described by May.[6]

These diazonium chloride antimony trichloride complex salts are very stable and their formation is almost quantitative. As the product formed here, $CH_3CONHC_6H_4N_2Cl.SbCl_3$, is somewhat soluble in hydrochloric acid, the yield of this reaction is only about 90%.

This compound was filtered off, suspended in water and the suspension was added slowly to a 10% solution of sodium hydroxide heated to 90°, the solution being stirred mechanically. This method gave good results and the time of the procedure was considerably shortened. The evolution of nitrogen was comparatively rapid and the foam formed on each addition of the molecular compound subsided rapidly. The addition usually takes from one to two hours. After all the suspension was added, the solution was heated at the same temperature until a test filtered portion precipitated and redissolved in excess dilute hydrochloric acid. The solution was then nearly neutralized and saturated with carbon dioxide to precipitate impurities. It was filtered, and from the filtrate stibanilic acid, $NH_2C_6H_4SbO_3H_2$, precipitated by acetic acid, the acetyl group having been removed during the procedure. The crude stibanilic acid is removed from the liquid by centrifuging and is then ready for use. The yield varies but is generally about 50%, figured on the crude acid.

The breaking down of the molecular compound was also effected by ammonium hydroxide, sodium carbonate solutions and solutions of sodium hydroxide of varying strengths. The effect of copper powder, mild oxidizing agents, etc., was also tried with no success.

As the crude product has been found to be sufficiently pure for our purpose, it has been used without further purification. This has been

[4] Ger. pat. 254,421 (1912); 261,825 (1913); 220,488 (1913).

[5] Schmidt, *Ann.*, **421**, 174 (1920).

[6] May, *J. Chem. Soc.*, **101**, 1037 (1912).

particularly fortunate as stibanilic acid is far from being a stable compound, decomposing rapidly on keeping. For this reason, we have prepared our acid as we needed it and have made no attempt to keep it in solid form.

Preparation of Dyes

The second and third series of dyes were made after the same general method described previously.[1] For the preparation of the second series, substituted anilines were utilized as intermediates. Dimethyl-, diethyl-, dipropyl- and dibutylaniline were used but, unfortunately, compounds were formed only by dimethyl- and diethylaniline. These were made as follows.

A calculated amount of stibanilic acid was diazotized with 10% sodium nitrite solution in the customary manner. This was poured into hydrochloric acid solutions containing calculated quantities of dimethyl- and diethylaniline. These solutions were allowed to stand for some time, filtered and made alkaline. The alkaline solutions were clear at first but on standing precipitates formed. These proved to be the *p*-nitroso derivatives of the substituted anilines used. Their formation is due to an excess of nitrous acid in the diazotized stibanilic acid solution, which is difficult to avoid. The solutions were filtered from these impurities and the acid dyes were precipitated by hydrochloric acid. These precipitates were collected by centrifuging and were dried and then washed with hydrochloric acid to remove any phenylstibinic acid or similar by-product and any excess of the intermediate.

The resulting *p*-dimethyl- (VII) and *p*-diethylamino-azobenzene (VIII) were, respectively, dull brown and dull purple in color and both were insoluble in water and organic solvents and soluble in sodium and ammonium hydroxide.

Anal. Calcd. for $C_{14}H_{16}O_3N_3Sb$: Sb, 30.7. Found: 29.9.
Calcd. for $C_{16}H_{20}O_3N_3Sb$: Sb, 28.7. Found: 27.5.

The third series of dyes has been prepared after the following general scheme. Calculated amounts of diazotized stibanilic acid were added to slightly alkaline solutions of various substituted naphthalene sulfonic acids in theoretical amounts. These solutions were warmed and allowed to stand for several hours and then filtered. The filtered solutions were evaporated to small volume on a water-bath. At this point the procedure of preparation differed according to the nature of the dye. If it was possible to precipitate by acid, the solution was acidified, the precipitate collected by centrifuging and dried. The dried, powdered precipitate was thoroughly washed with hydrochloric acid and then with water. This removed any excess intermediate or breakdown products of stibanilic acid. It was again dried and analyzed for antimony. The following compounds were prepared by this method.

Diazotized stibanilic acid was coupled with Neville and Winther's acid, J-acid and amino R salt, respectively, to give compound XIII, dark red; compound XIV, light red brown; and compound XVI, dark brown. Compounds XIII and XIV were insoluble in water. Compound XVI was moderately soluble. All were insoluble in organic solvents but soluble

in sodium and ammonium hydroxides and concentrated sulfuric acid. Sodium salts of all were prepared.

Analyses of the dyes and their sodium salts.

(XIII) Calcd. for $C_{16}H_{13}O_7N_2SSb$: Sb, 24.4. Found: 22.6.
Calcd. for $C_{16}H_{10}O_7N_2SNa_3Sb$: Sb, 21.3. Found: 20.1.
(XIV) Calcd. for $C_{16}H_{14}O_7N_3Sb$: Sb, 23.7. Found: 22.4
Calcd. for $C_{16}H_{11}O_7N_3Na_3Sb$: 21.0. Found: 19.4.
(XVI) Calcd. for $C_{16}H_{14}O_9N_2S_2Sb$: Sb, 21.0. Found: 20.3.
Calcd. for $C_{16}H_{10}O_9N_3Na_4Sb$: Sb, 18.2. Found: 19.5.

The preparation of compounds of Series III which could not be precipitated with acid occasioned much difficulty. By concentrating to very small volume and making the solution very strongly acid with hydrochloric acid, small yields could be obtained. Several methods were attempted in order to isolate the dye. A concentrated solution of the dye was treated with a saturated solution of sodium chloride in an effort to salt out the dye. This method was not very satisfactory as the precipitate contained much salt and, in addition, a large amount of the dye remained in solution. Fractional precipitation from alcohol-water mixture gave fair results but the method was very tedious and the yields were poor.

The method finally adopted was the precipitation of the barium salt of the dye by treating the solution with a solution of barium chloride. The barium salts of the dyes separate out as apparently amorphous precipitates. These precipitates are collected by filtering the solution or by centrifuging, washed thoroughly and dried. Fortunately, the barium salts of the intermediates used to make these dyes are relatively soluble in water and if any of them precipitate with the dye, washing with water removes them. The dried compounds were weighed and then suspended in water. To these suspensions a dilute solution containing sufficient sodium sulfate to precipitate the barium was added. In fact, it is better to add a slight excess of sodium sulfate to prevent the presence of the highly toxic barium ion in the final product. The mixtures were warmed and stirred vigorously. Barium sulfate was thrown down and the sodium salts of the dyes went into solution. A small amount of the dyes is adsorbed on the precipitates, coloring them highly. The barium sulfate was filtered off and the resulting solutions of the sodium salts of the various dyes were evaporated to dryness either on a water-bath or "in vacuo." It is better to dry in a vacuum. Each was tested for barium to insure its absence.

Diazotized stibanilic acid was coupled with H-acid, R-salt and chromotrope, respectively, to give compounds XII, brilliant purple; XV, orange brown; and XVII, purple. These are all soluble in water, insoluble in organic solvents and soluble in concentrated sulfuric acid. They decompose on heating.

Analyses:

(XII) Calcd. for $C_{16}H_{10}O_{10}N_3S_2Na_4Sb$: Sb, 17.9. Found: 19.5.
(XV) Calcd. for $C_{16}H_9O_{10}N_2S_2Na_4Sb$: Sb, 18.2. Found: 17.2.
(XVII) Calcd. for $C_{16}H_{10}O_{11}N_2S_2Na_4Sb$: Sb, 17.8. Found: 16.9.

For comparison, the analogs of all of these dyes were prepared from diazotized sulfanilic acid and the same intermediates. These were isolated as the sodium salts. They have been utilized as dyes for the dyeing of woolen skeins and have also been tested pharmacologically on rats infected with *Trypanosoma equiperdum*.

Miscellaneous

Several antimony compounds which do not belong to any of these classes of dyes have been prepared. The general method of preparing these derivatives has been the application of the diazo reaction for introducing the stibinic acid group. As a consequence, various aromatic amines have been the starting-point for all of the subsequent syntheses.

May found that a double salt of diazotized sulfanilic acid and antimony trichloride could not be prepared, due in all probability to the strong acidic character of the sulfonic acid group. This has been confirmed and also has been found to apply to diazotized anthranilic acid. That the formation of the double salt is prevented by the presence of the acidic groups seems to be indicated strongly by the fact that we have been able to prepare antimony trichloride molecular double salts with diazotized methyl anthranilate and with *p*-aminobenzoic acid. The latter compound is formed with some difficulty. These double salts have been broken down with sodium hydroxide to form the corresponding stibinic acid derivatives.

Antimony compounds have also been prepared from diazotized benzidine, *p*-aminodimethylaniline and *p*-aminodiethylaniline.

The method of preparation has been the same in every case. Tenth molar proportions of the amine and antimony trioxide were dissolved in 100 cc. of water and 60

TABLE I

ANTIMONY COMPOUNDS

Molecular compound	Stibinic acid	Analysis, % Sb, Calcd.	Found
$(CH_3)_2NC_6H_4N_2Cl.SbCl$—white crystals[a]	$(CH_3)_2NC_6H_4SbO_3H_2$—white	41.7	40.8
$(C_2H_5)_2NC_6H_4N_2Cl.SbCl_3$—greenish crystals[a]	$(C_2H_5)_2NC_6H_4SbO_3H_2$—white	38.0	36.1
$C_6H_4N_2Cl.SbCl_3$ | $C_6H_4N_2Cl.SbCl_3$—light yellow crystals	$C_6H_4SbO_3H_2$—yellow	49.1	54.0
$C_6H_4(COOCH_3)(N_2Cl.SbCl_3)$—white crystals	$C_6H_4(COOCH_3)(SbO_3H_2)$—white	39.3	42.1

[a] These crystals were contaminated with the *p*-nitroso di-alkyl aniline, which gave them a greenish tinge.

cc. of concentrated hydrochloric acid. This solution was cooled to 0° and a 10% water solution of 6.9 g. of sodium nitrite was added through a capillary at the bottom of the beaker, the contents being stirred rapidly. The crystalline double salts, being comparatively insoluble in hydrochloric acid of this concentration, settled out. These were filtered off and suspended in water and this suspension was added slowly to 10% sodium hydroxide at 90°. During the addition the mixture was stirred mechanically and a brisk evolution of nitrogen took place. After this reaction was over, the by-products were precipitated by making the solution almost neutral and passing in carbon dioxide. The sodium salts of the various aromatic stibinic acids were then precipitated by adding alcohol. These salts were obtained by filtering and were dissolved in water and the acid form of the compounds precipitated by dilute acetic acid.

These products were insoluble in water and the usual organic solvents but soluble in sodium and ammonium hydroxides. They decomposed on heating (see Table I).

Analytical

The analyses of these compounds occasioned some difficulty and many methods were tried. The method of Schmidt with a slight modification was used with fair success. After the sample had been burned down with 5 g. of sodium bisulfate and 10 cc. of concentrated sulfuric acid and diluted tartaric acid was added, sulfur dioxide was passed through to insure the reduction of all pentavalent antimony. The excess sulfur dioxide was removed by boiling, preferably in an atmosphere of carbon dioxide. The solution after cooling was neutralized with sodium bicarbonate and then titrated with 0.1 *N* iodine solution.

The method of Macallum works satisfactorily for the water-soluble compounds.

The use of the Parr bomb for oxidation of the sample with sodium peroxide was attempted. The subsequent procedure was: acidification with hydrochloric acid, filtering, adding cooled solution to a solution of 5 g. of potassium iodide in 100 cc. of water and titrating liberated iodine with 0.1 *N* thiosulfate. The results, using this method, were low.[7,8,9,10]

Pharmacological

All the derivatives of these three series of antimony dyes have been tested against a virulent strain of *Trypanosoma equiperdum*. The white albino rat has been used as a test animal. One per cent. solutions of the dyes were utilized and the results are summarized in Table II.

A more complete report on the pharmacology of these compounds will appear elsewhere.

Antimony and the Color of Dyes

In view of the fact that the compounds described are dyes, it was thought of interest to ascertain the effect of the antimony group as an auxochrome.

[7] Rohmer, *Ber.*, **34**, 1565 (1901).

[8] Fargher and Gray, *J. Pharmacol.*, **18**, 356 (1921).

[9] Macallum, *J. Soc. Chem. Ind.*, **42**, 468T (1923).

[10] Schmidt, *Ann.*, **421**, 244 (1920).

TABLE II

GENERAL SUMMARY

Compound	Sb content	Toxic dose, g. per kg.	Effective dose	Chemotherapeutic index	Attempts	Cures	Remarks
I to VIII	...	...	...	...	3 each	0	Practically non-toxic. All compounds of these series useless.
XII	19.5	0.3	0.05	6–7	14	8	
XIII	20.1	.3	.1	...	5	0	Effective for mild infection.
XIV	19.4	.4	.09	4–5	8	3	Cures with treatment give promise.
XV	17.3	.4	.1	...	5	0	Effective with mild infection.
XVI	17.5	.4	.08	4–5	10	6	
XVII	16.9	.4	...	...	5	0	Effective with mild infection.

Accordingly, dyes were made for this purpose by coupling diazotized stibanilic acid with various intermediates, and for comparison diazotized sulfanilic acid was coupled with the same intermediates. The intermediates used for this purpose were as follows: H-acid, Neville and Winther's acid, J-acid, R-salt, G-salt, Armstrong's acid, Epsilon acid, Schaeffer's salt, chromotrope, phenol, chlorophenol, *o*-cresol, *o*-nitrophenol and salicylic acid.

Woolen and cotton skeins, five grams in weight, were utilized for application of the dyes. Solutions of each dye were prepared in such a manner as to give a 1% dyeing, based on the weight of the skein. Calculated quantities of solutions containing known amounts of diazotized stibanilic and sulfanilic acids, respectively, were pipetted into beakers containing dilute solutions of the requisite amounts of the various intermediates, these solutions being alkaline with sodium carbonate. The technique of the ensuing manipulations is that described by Waldron and Reid[11] for the dyeing of woolen and cotton skeins, respectively.

It was found that the antimony dyes had the property of being direct

TABLE III

COLOR OF DYES

Intermediate	Auxochrome groups —SO_2H (wool)	—SbO_3H_2 (wool)	—SbO_3H_2 (cotton)
H-acid	Bordeaux	Reddish violet	Light violet
Neville and Winther's acid	Bright reddish orange	Light red	Dull pink
R-salt	Light dull reddish orange	Dull yellowish red	Pale dull reddish orange
G-salt	Very light buff	Pale dull yellowish orange	Very pale, dull reddish orange
Epsilon acid	Bright yellowish brown	Bright yellowish brown	Dull reddish orange
Chromotrope	Cerise	Dull reddish violet	Light bluish violet
Schaeffer's salt	Dull reddish yellow	Orange	Very dull reddish orange
Chlorophenol	Dull yellow	Light dull yellow	Dull cream
Cresol	Greenish yellow	Dull yellow	Cream

[11] Waldron and Reid, THIS JOURNAL, **45**, 2412 (1923).

dyes for cotton, whereas the sulfanilic acid derivatives were not fast to cotton and were completely washed out.

The colors of the resulting skeins indicate that the antimony group (the stibinic acid group) is more strongly auxochromic in character than is the sulfonic acid group. Table III lists the colors of the various dyes.

Our thanks are extended to Dr. R. E. Rose, of the Technical Laboratory of E. I. du Pont de Nemours and Co., who kindly furnished the skeins for this work and the color designations of the finished products, and also to Mr. C. Slagle of this Laboratory who assisted in preparing the dyed skeins.

Summary

1. The method of preparing stibanilic acid has been improved.
2. Three series of azo dyes containing antimony have been made and tested against trypanosomiasis in the white rat.
3. The effect of antimony on color has been ascertained.
4. Several other antimonials have been described.

BALTIMORE, MARYLAND

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF MOUNT HOLYOKE COLLEGE]

VARIOUS EFFECTS PRODUCED BY THE ACTION OF LIGHT UPON THE ISOMERIC MODIFICATIONS OF CERTAIN POLYPEPTIDE-HYDANTOINS

BY DOROTHY A. HAHN AND JANET EVANS

RECEIVED JULY 23, 1927 PUBLISHED NOVEMBER 5, 1927

As a result of the investigation of a fairly large number of pairs of geometrical isomers corresponding to different derivatives of benzal[1] and anisalhydantoin,[2] the interesting observation has frequently been made that during the process of preparation and purification of these substances, minute quantities of very high-melting and very insoluble compounds were formed. These substances were markedly different in their properties from any of the major products taking part in the particular transformations but were never formed in quantities sufficient to admit of a study of their properties or even of their analysis. Recent work on the isomeric modifications of methyl-N-3-methylbenzalhydantoin-N-1-acetate and of the corresponding acids,[1] has at last made it possible to arrive

I $CH_3OOCCH_2N(CO)(CO)CH_3N-C=CHC_6H_5$

(a) m. p., 65.5–66.5°
(b) m. p., 98.5–99.5°

II $HOOCCH_2N(CO)(CO)CH_3N-C=CHC_6H_5$

(a) m. p., 186.5–187.5°
(b) m. p., 198.5–199.5°

[1] Hahn and Evans: The preparation and properties of these substances have been described in a paper which was received by THIS JOURNAL, July 23, 1927.

[2] Hahn and Gilman, THIS JOURNAL, **47**, 2953 (1925).