TLC Sensitivity of Six Modifications of Dragendorff's Reagent

LINDA B. RUBIA and RALPH GOMEZ ˣ

Abstract □ The relative sensitivities of six modifications of Dragendorff's reagent were measured on TLC plates by spectrodensitometry, using compounds containing specific functional groups. A correlation between the structures of the compounds reactive to Dragendorff's reagents and the sensitivities of the reagents was made. Explanations for the variations in sensitivities between different modifications of Dragendorff's reagent are given.

Keyphrases □ Dragendorff's reagent—several modifications, effect on relative TLC sensitivities of compounds containing various functional groups □ TLC—effect of modifications of Dragendorff's reagent on relative sensitivities of compounds containing various functional groups □ Structure–activity relationships—compounds containing various functional groups, effect of modification of Dragendorff's reagent on relative TLC sensitivities

Dragendorff's reagent is a commonly used spray reagent for the detection of nitrogen-containing compounds in the TLC analysis of pharmaceutical products (1–3). Its sensitivity of detection of different compounds, relative ease of preparation, and stability are some reasons for its extensive use. Dragendorff's reagent has been known for over a century (4) and has been used as a TLC reagent for over 25 years.

Many modifications have been suggested to increase its sensitivity, specificity, and stability (5–8). The purpose of this investigation was to determine whether any modification offered advantages over the others and whether one could be used as a general spray reagent for the detection of nitrogen-containing active pharmaceuticals.

The sensitivities of six modifications of Dragendorff's reagent were measured on TLC plates by spectrodensitometry, using compounds containing specific basic functional groups. The sensitivities of the modifications were determined, and their reactivity to the functional groups was studied. The reactions that explain the differences in sensitivities between the reagents are discussed.

EXPERIMENTAL

Equipment—A spectrodensitometer[1] equipped with a monochromator was used. The wavelength was 500 nm, and the signal was fed into a properly attenuated recorder.

Materials and Reagents—*Plates*—Silica gel G was slurried with water and coated on 20 × 20-cm glass plates using a mechanically operated TLC coating apparatus[2]. The plates were dried at 40° overnight; the average layer thickness was 0.25 mm.

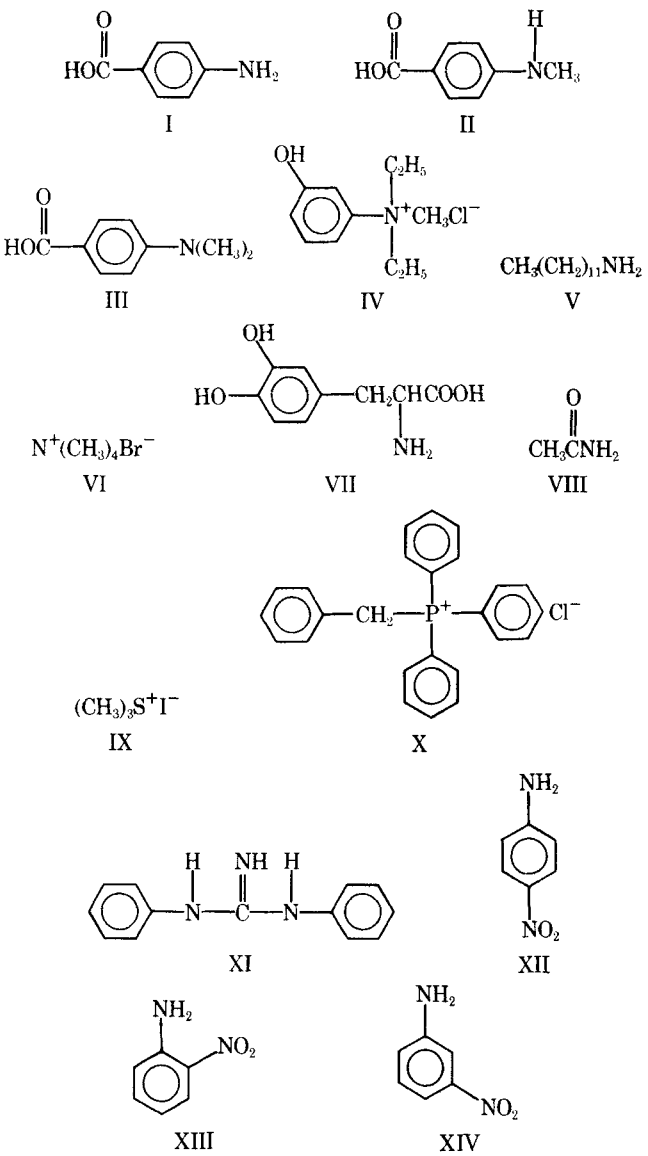
Solutions—The following solutions were prepared: 1% (w/v) aqueous sodium nitrite, 40% (w/v) aqueous potassium iodide, 1.7% (w/v) bismuth nitrate in 20% (v/v) acetic acid, and 10% (w/v) aqueous sulfuric acid.

Acid-Modified Dragendorff's Reagent (Reagent A)—Potassium iodide solution, 100 ml, was added to 100 ml of bismuth nitrate solution, and the mixture was diluted to 1 liter with 10% (w/v) sulfuric acid.

Peroxide–Acid-Modified Dragendorff's Reagent (Reagent B)—To 300 ml of Reagent A, 0.6 ml of 30% hydrogen peroxide was added.

Iodine–Acid-Modified Dragendorff's Reagent (Reagent C)—To 300 ml of Reagent A, 6 g of iodine crystals was added. Then the mixture was shaken for 30 min.

[1] Schoeffel model SD 3000.
[2] Camag model 21-602.



Acid-Modified Dragendorff's Reagent Oversprayed with Sodium Nitrite (Reagent D)—After spraying the TLC plate with Reagent A, the plate was lightly sprayed with sodium nitrite solution.

Peroxide–Acid-Modified Dragendorff's Reagent Oversprayed with Sodium Nitrite (Reagent E)—After spraying the TLC plate with Reagent B, the plate was lightly sprayed with sodium nitrite solution.

Iodine–Acid-Modified Dragendorff's Reagent Oversprayed with Sodium Nitrite (Reagent F)—After spraying the TLC plate with Reagent C, the plate was lightly sprayed with sodium nitrite solution.

Iodine Solution—To 10 ml of 40% potassium iodide solution, 2 g of iodine crystals was added. The mixture was diluted to 100 ml with 10% (w/v) sulfuric acid and mixed well.

Samples and Standard—The compounds studied were: 4-aminobenzoic acid (I), 4-(*N*-methylamino)benzoic acid (II), 4-(*N*,*N*-dimethylamino)benzoic acid (III), edrophonium chloride (IV), dodecylamine (V), tetramethylammonium bromide (VI), levodopa (VII), acetamide (VIII), trimethylsulfonium iodide (IX), benzyltriphenylphosphonium chloride (X), diphenylguanidine (XI), 4-nitroaniline (XII), 2-nitroaniline

Table I—Relative Response to Dragendorff's Reagents

Compound	Reagent A	Reagent B	Reagent C	Reagent D	Reagent E	Reagent F
I	0.2	0.7	0.9	1.0	0.8	1.0
II	0.2	0.9	1.6	1.2	1.2	1.6
III	0.4	1.4	2.8	2.4	2.5	2.9
IV	0.9	2.8	3.8	3.9	3.9	4.4
V	0.6	1.0	1.2	1.4	1.3	2.6
VI	1.7	3.7	4.2	3.8	3.6	4.4
VII	0.0	0.0	0.0	0.0	0.0	0.0
VIII	0.0	0.0	0.0	0.0	0.0	0.0
IX	0.9	2.7	3.4	3.5	4.3	4.1
X	1.4	3.5	4.7	4.5	4 8	5.1
XI	0.5	2.1	2.9	3.1	4.1	4.9
XII	0.2	0.9	1.2	0.9	1.6	1.8
XIII	0.4	0.5	0.7	0.4	0.8	0.5
XIV	0.1	0.2	0.7	0.2	0.6	0.8

(XIII), and 3-nitroaniline (XIV). Solutions of the compounds were prepared in either chloroform or chloroform–methanol (92:8) at a concentration of 0.6 mg/ml. The standard was 1% (w/v) methanolic thymol blue (thymolsulfonphthalein sodium salt).

Procedure—A 20 × 20-cm TLC plate was scored into 20 parallel 1-cm lanes. With a syringe, alternate lanes were spotted with 5 μl of sample solution. The spots were thoroughly dried, and the plates were sprayed evenly with 10 ml of Dragendorff's reagent. Then the plates were immediately covered with a 20 × 20-cm piece of clear glass and scanned. Five determinations on five separate plates were performed for each sample. On each plate, 2 μl of thymol blue solution was spotted. The areas of all spots were measured relative to the area of the thymol blue spot.

RESULTS AND DISCUSSION

The relative sensitivities of 14 compounds sprayed with six modifications of Dragendorff's reagent are summarized in Table I. In most cases, Reagent F proved to be the most sensitive, followed by Reagent C. Reagents A and B had the lowest sensitivities, while Reagents D and E had intermediate sensitivities.

An explanation for the observed order of sensitivities may be found in the preparation of the reagents since the relative sensitivity of each reagent can be roughly correlated to the amount of available iodine. To illustrate this correlation, IV–VI and IX–XI were spotted on TLC plates, sprayed with a solution of iodine of an equal concentration, and prepared in the same solvent as Dragendorff's reagent. The relative responses for IV, V, VI, IX, X, and XI were 4.0, 1.7, 4.1, 3.3, 5.3, and 3.9, respectively. These results show that the relative areas were similar to those obtained for Reagents C and F and greater than those obtained for Reagents A and B, which do not contain added iodine, and indicate that the high sensitivity of Reagents C and F is due largely to their iodine content.

The contribution of sodium nitrite to the total sensitivity of the reagent can be seen from the data in Table I. Comparison of the data for Reagents A and D shows that the latter was more sensitive. Similar results are seen when Reagents B and E and C and F are compared. The difference between reagents in each pair is that Reagents E and F are oversprayed with sodium nitrite solution while B and C are not. This increase in sensitivity, therefore, is attributed to sodium nitrite overspraying. As Fike (9) proposed, sodium nitrite releases iodine from the excess iodide in the reagents, which enhances the sensitivity.

A possible reaction that explains the role of sodium nitrite in the reagent is shown in Scheme I (10).

$$2NO_2^- + 2I^- + 4H^+ \rightarrow I_2 + 2NO + 2H_2O$$

Scheme I

The contribution of hydrogen peroxide to the sensitivity of the reagent can be seen by inspecting Table I, which shows that Reagent B was more reactive than Reagent A. The only difference in the two is that Reagent B has added hydrogen peroxide. Hydrogen peroxide reacts with excess iodide in the reagent to liberate iodine, similar to sodium nitrite.

A possible reaction is shown in Scheme II (10).

$$H_2O_2 + 2H^+ + 2I^- \rightarrow I_2 + 2H_2O$$

Scheme II

Reagent 3 cannot be expected to be as sensitive as the other reagents containing iodine or oversprayed with sodium nitrite, since the amount of hydrogen peroxide added is small and, therefore, the amount of iodine that can form and be available for reaction is small.

The data show that addition of crystalline iodine or formation of iodine by reaction with sodium nitrite or hydrogen peroxide increased the sensitivity of each reagent over its noniodine-containing counterpart.

Table I shows that IV, VI, IX, and X were the most reactive with Dragendorff's reagents, while III and XI were close behind in reactivity. These reactive compounds are quaternary nitrogen and phosphorus salts and tertiary sulfur salts. Compounds III and XI are tertiary amines, which can be easily converted to quaternary amine salts by reaction with the excess sulfuric acid in the reagents. Presumably, reaction with the reagent then proceeds by the same mechanism as with the salts.

Compounds I, V, XII, XIII, and XIV, all primary amines, had a relatively low reactivity with the reagents, although V, the aliphatic primary amine, had a higher sensitivity than the other four compounds.

Compounds VII and VIII, an amino acid and amide, respectively, did not react with any of the six modifications of the reagent.

For a homologous series, I–III, reactivity increased from primary to secondary to tertiary amine; with *ortho*-, *para*- and *meta*-substituted nitroanilines, reactivity was generally poor and the order of reactivity varied with the reagent.

The role of light and air on the stability of plates sprayed with Reagent C was investigated. Duplicate plates were spotted with 3 μg of IV, sprayed with Reagent C, and immediately covered with a 20 × 20-cm piece of plain glass. The sides were sealed with tape. One plate was left exposed to room light, and the second was kept in the dark. Both plates were periodically scanned on the densitometer. After 5 hr, the relative areas of the spots were fairly constant for both plates.

The experiment was repeated. This time the plates were left uncovered. After several hours, the spots had faded a great deal. However, the decrease was approximately the same for the plate exposed to light and the plate kept in the dark. This result indicates that the spot on the plate is not light sensitive but appears to be sensitive to air.

Since Dragendorff's reagent is used extensively in the pharmaceutical industry for the detection of nitrogen-containing drug substances on TLC plates, these results should aid in improving the detection limits of these active drug substances and their degradation products on TLC plates.

REFERENCES

(1) "Thin-Layer Chromatography," 2nd ed., E. Stahl, Ed., Academic, New York, N.Y., 1969, p. 873.

(2) J. G. Kirchner, "Thin-Layer Chromatography," Interscience, New York, N.Y., 1967, p. 160.

(3) K. Randerath, "Thin-Layer Chromatography," Academic, New York, N.Y., 1964, p. 74.

(4) G. Dragendorff, *Russ. J. Pharm.*, **5**, 85 (1866). *Ibid.*, **6**, 663 (1867).

(5) H. M. Bregoff, E. Roberts, and C. C. Deliveche, *J. Biol. Chem.*, **205**, 565 (1953).

(6) R. Munier and M. Machebouf, *Bull. Soc. Chem. Biol.*, **31**, 1144 (1949). *Ibid.*, **33**, 846 (1951).

(7) R. Munier, *Bull. Soc. Chem. Biol.*, **35**, 1225 (1953).

(8) H. Thies and F. W. Reuther, *Naturwissenschaften*, **41**, 230 (1954).

(9) W. W. Fike, *Anal. Chem.*, **38**, 1697 (1966).

(10) G. H. Ayres, "Quantitative Chemical Analysis," Harper, New York, N.Y., 1958, p. 471.

ACKNOWLEDGMENTS AND ADDRESSES

Received January 3, 1977, from the *Research Products Section, Quality Control Department, Hoffmann-La Roche Inc., Nutley, NJ 07110.*

Accepted for publication January 31, 1977.

The authors acknowledge the assistance of the Scientific Literature Department of Hoffmann-La Roche Inc.

[x] To whom inquiries should be directed.