Stabilization of Homatropine Methylbromide–Iodobismuthate Complex in Solution for Quantitative Analysis of Homatropine Methylbromide

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Abstract ☐ The colorimetric reaction between homatropine methylbromide (HMB) and the Dragendorff reagent was investigated as a means of quantitative analysis for HMB. The reaction was found to be unstable under normal laboratory lighting conditions. Stabilization of the colorimetric reaction was achieved by the addition of methanol to an acetone solution of the HMB–iodobismuthate complex in the ratio of 1:1. The stabilization was accompanied by a hypsochromic shift from 474 to 382 mμ. The stabilized colorimetric reaction follows the Beer–Lambert law in the range of concentrations of HMB studied for this investigation. The sensitivity of the method was found to be 0.1 mg. of HMB under the experimental conditions described. However, with slight modifications, this method should be useful for much smaller concentrations of HMB.

Keyphrases ☐ Homatropine methylbromide analysis—reaction mechanism ☐ Iodobismuthate complex stabilization—homatropine methylbromide analysis ☐ Colorimetric reaction—stabilization

For many years, the Dragendorff reagent has been used as a qualitative test reagent for alkaloids. However, it is only recently that the applications of this reagent for quantitative analysis have been reported (1–4). Pfordte (1) used the Dragendorff reagent, as modified by Trabert (5), in the paper chromatographic analysis of atropine, hyoscyamine, and scopolamine. The orange-colored alkaloid spot was eluted from the sprayed paper chromatographic strips with acetone, and the absorbance of this solution was measured at 400 mμ, because the maximum at 475 mμ faded quickly. Paris and Saint-Firmin (2, 3) employed a thin-layer chromatographic method instead of the paper chromatographic method for quantitative analysis of hyoscyamine and scopolamine bromide with the Dragendorff reagent, as modified by Trabert (5). They used 365 mμ as the analytical wavelength and noted that the colorimetric solutions were stable in the dark for 30 min. (2) to several days (3).

The present investigation was initiated with the objective of studying the colorimetric reaction of homatropine methylbromide and the Dragendorff reagent as a means of quantitative analysis for homatropine methylbromide (HMB). In the earlier stages of investigation, it was discovered that the colorimetric reaction was unstable, so efforts were made to stabilize the colorimetric reaction in order to achieve a more reliable method of analysis.

EXPERIMENTAL

Reagents—Acetone and methanol, spectroquality, Baker; homatropine methylbromide NF; 5% sulfuric acid; Dragendorff reagent, as modified by Munier and Macheboeuf (6). Ten milliliters of glacial acetic acid and 40 ml. of water are added to 850 mg. of bismuth subnitrate. To 40 ml. of this slurry, 40 ml. of 40% aqueous potassium iodide solution, 125 ml. of glacial acetic acid, and 250 ml. of water are added.

Table I—Stability of HMB–Iodobismuthate Complex in Solution

Solvent Acetone: Methanol	$\lambda_{\text{max.}}$ (mμ)	Absorbance at Indicated Time, min. 0	15	30	60
100:0	474	0.438	0.453	0.406	0.260
90:10	464	0.448	0.420	0.276	0.199
80:20	459	0.420	0.400	0.350	0.295
70:30	449	0.351	0.329	0.301	0.285
60:40	380	0.497	0.500	0.502	0.634
50:50	382	0.505	0.503	0.502	0.501
40:60	383	0.492	0.488	0.487	0.487
30:70	387	0.460	0.427	0.449	0.756

Method—An accurately weighed amount of homatropine methylbromide was dissolved in distilled water to yield a 0.25-mg./ml. solution. Five milliliters of this solution (equivalent to 1.25 mg. of homatropine methylbromide) was transferred to a 100-ml. beaker, and 2 drops of 5% sulfuric acid and 1 ml. of freshly prepared modified Dragendorff reagent were added. The resulting precipitate was allowed to stand for 1 hr. to facilitate filtration. (The precipitation is quantitative and cannot be readily extracted into any of the common immiscible organic solvents; therefore, filtration is used.) The precipitate was filtered through a fine-porosity sintered-glass funnel with the aid of vacuum. The precipitate was dried by suction and dissolved in acetone directly in the sintered-glass funnel and the solution was collected in a 50-ml. volumetric flask containing a known volume of methanol. The volume was made up with acetone. The resulting solutions were scanned with a recording spectrophotometer[1] in 1-cm. cells.

RESULTS AND DISCUSSION

The Dragendorff reagent, as modified by Munier and Macheboeuf (6), was used for this investigation. This reagent is commonly used in these laboratories as a spray reagent for TLC plates (7) and in that respect, it is similar to that used by previous workers (1–3).

The reaction product of an alkaloid and the Dragendorff reagent has been called alkaloid–iodobismuthate complex (1, 8, 9) and will be so referred to in this paper. The HMB–iodobismuthate complex is obtained as an orange precipitate when HMB is reacted with the Dragendorff reagent, as modified by Munier and Macheboeuf (6). This precipitate, when solubilized in acetone, yields an orange-colored solution with a maximum at 474 mμ. Due to some change in the iodobismuthate complex, the absorbance at 474 mμ decreases rapidly with a resulting shift of the maximum to 362.5 mμ. From investigations in these laboratories, the stability of the maximum at 362.5 mμ under regular laboratory lighting conditions, appears to be questionable. [The complex obtained with Dragendorff reagent, as modified by Trabert (5), was also found to be unstable.] Therefore, stabilization of the iodobismuthate complex was considered desirable so that quantitation could be achieved by the use of the complex itself rather than an unknown by-product.

Studies were conducted with several mixtures of solvents to determine a suitable solvent system which would offer greatest stability characteristics for the complex.

Stabilization of the HMB–iodobismuthate complex could be achieved by the addition of methanol to the acetone solution of the complex in the ratio of 1:1. The resulting 50% acetone–methanol

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[1] Cary 15 recording spectrophotometer, Cary Instruments, Monrovia, Calif.

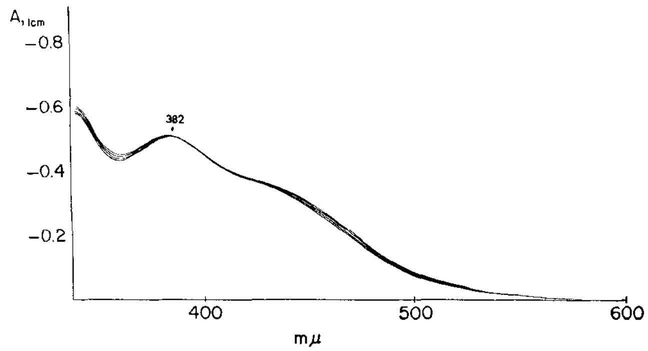

Figure 1—*Stability of homatropine methylbromide–iodobismuthate complex in 50% acetone–methanol solution (recorded at 15-min. intervals over a period of 1 hr.).*

Table II—Relationship between Concentration of HMB and Absorbance

Concentration of HMB, mg.	Absorbance at 382 mμ
0.1	0.035
0.2	0.070
0.4	0.152
0.6	0.245
1.0	0.390
1.2	0.480
1.4	0.540
1.6	0.600
1.8	0.680

solution was stable for at least 1 hr. in normal laboratory lighting conditions. This time period was considered adequate even if a large number of samples have to be analyzed at the same time; therefore, longer time periods were not checked.

The stabilization was accompanied by a hypsochromic shift from 474 to 382 mμ. Stabilization of the complex could be demonstrated by allowing the acetone–methanol solution to stand for half an hour, evaporating the solvents, dissolving the residue in acetone, and observing the return of the maximum at 474 mμ. This was not the case with solution of the complex in acetone alone, thereby suggesting a definite change in the complex. Table I shows the hypsochromic shifts caused by the addition of various amounts of methanol to the acetone solution of HMB–iodobismuthate complex. The stability of these solutions under normal laboratory lighting conditions, as indicated by change in absorbance readings at $\lambda_{max.}$, is also shown in Table I. (The reagent blank readings were found to be negligible.)

From these results, it is apparent that stabilization can be achieved when 50 to 60% methanol is present in the acetone–methanol solution with greatest stability being indicated for 50% methanol content. The stability of 50% acetone–methanol solution of the HMB–iodobismuthate complex over a 1-hr. period can be seen in Fig. 1. These data also show that greater sensitivity is obtained with 50% acetone–methanol solution. This was confirmed by studying acetone–methanol solutions of the HMB–iodobismuthate complex containing 55.0, 52.5, 47.5, and 45.0% of methanol. Therefore, it is logical to conclude that of the various acetone–methanol solutions investigated, 50% acetone–methanol solution offers the greatest stability and sensitivity for the quantitation of iodobismuthate complex.

Table II shows that the colorimetric reaction follows the Beer–Lambert law from 0.1 to 1.8-mg. concentrations of HMB. Higher concentrations than 1.8 mg. were not investigated. Lower workable limits were found to be 0.1 mg. under the experimental conditions described. However, with slight modifications such as final dilution volume and the size of the spectrophotometric cell, it should be possible to work with much smaller concentrations of HMB.

These investigations have shown that Dragendorff reagent, as modified by Munier and Macheboeuf (6), can prove to be a useful reagent for quantitative analysis of homatropine methylbromide. The colorimetric reaction is stable and sensitive to small concentrations of HMB. Furthermore, the colorimetric reaction should prove useful for quantitative analysis of other alkaloids and compounds reactive with the Dragendorff reagent.

CONCLUSIONS

The Dragendorff reagent, as modified by Munier and Macheboeuf (6), has been found to be a useful reagent for quantitative analysis of homatropine methylbromide. The resulting homatropine methylbromide–iodobismuthate complex in acetone solution has been stabilized by the addition of methanol in the ratio of 1:1. The colorimetric solution obtained has a maximum at 382 mμ and is stable for at least 1 hr. under normal laboratory lighting conditions. The colorimetric reaction follows the Beer–Lambert law down to a 0.1-mg. concentration of HMB, under the working conditions described in this publication. With small modifications, this reaction could become sensitive for much smaller concentrations of HMB. Furthermore, it may be logically concluded that this method should prove useful for chemical compounds reactive with the Dragendorff reagent.

REFERENCES

(1) K. Pfordte, *J. Chromatogr.*, **21**, 495(1966).

(2) R. R. Paris and A. R. Saint-Firmin, *Compt. Rend.*, **264**, 825(1967).

(3) A. R. Saint-Firmin and R. R. Paris, *J. Chromatogr.*, **31**, 252(1967).

(4) K. Blaim and R. Cizewska, *Chem. Anal. (Warsaw)*, **12**, 761(1967).

(5) V. H. Trabert, *Pharm. Zentralhalle*, **93**, 462(1954).

(6) R. Munier and M. Macheboeuf, *Bull. Soc. Chim. Biol.*, **33**, 846(1951).

(7) L. G. Chatten, "Pharmaceutical Chemistry," vol. 1, Marcel Dekker, New York, N. Y., 1966, p. 387.

(8) M. Francois and L. G. Blanc, *Compt. Rend.*, **175**, 273(1922).

(9) M. Francois and L. G. Blanc, *Bull. Soc. Chim.*, **33**, 640(1923).

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