

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE AMERICAN MEDICAL ASSOCIATION.]

## A NEW COLOR REACTION FOR PAPAVERINE.

BY L. E. WARREN.

Received August 7, 1915.

In a study of the purity of commercial specimens of several of the opium alkaloids or their salts, a color reaction for papaverine ferricyanide was observed which, it is believed, has not hitherto been described. By modifications the test may be made to apply to the alkaloid, papaverine, or its commonly occurring salts, such as the chloride or sulfate, so that it seems probable that the reaction may prove of value in the identification of papaverine.

Papaverine is one of the minor alkaloids of opium. It occurs in Smyrna opium to the extent of about 0.8%, and in opium from other sources in amounts ranging from 0.2 to 1%. Papaverine is a weak base and is much less toxic than either codeine, morphine or thebaine. Formerly it was not used in medicine, but, owing to the recent pharmacological and clinical researches of Pal,<sup>1</sup> Popper,<sup>2</sup> Macht<sup>3</sup> and others, it has attracted some attention from clinicians.

Papaverine is distinguished from the other of the more important opium alkaloids by the very sparing solubility of its ferricyanide in water and by the deep rose color which is slowly developed if the alkaloid be dissolved in sulfuric acid which contains a little formaldehyde<sup>4</sup> (Marquis' reagent). A number of color reactions for papaverine are described in the older literature, but it has been shown by Pictet and Kramers<sup>5</sup> that most of these are not due to papaverine but to cryptopine which, until recently, was present as an impurity in most commercial papaverine. At present, however, commercial supplies of papaverine and its salts contain only traces of cryptopine.

Papaverine ferricyanide was first described by Plugge.<sup>6</sup> It is a pale lemon yellow compound which may be obtained as an amorphous precipitate by the addition with agitation of freshly prepared potassium ferricyanide solution to a faintly acidified, moderately dilute solution of a papaverine salt. Occasionally the precipitate separates in resin-like masses. From concentrated solutions narcotine and thebaine, if present, are also precipitated as pale yellow precipitates, the narcotine salt becoming green on exposure to the air. In dilutions above 1-500, papaverine

<sup>1</sup> *Wiener med. Wochenschr.*, 63, 1049 (1913); *Med. Times*, 42, 218 (1914).

<sup>2</sup> *Wiener klin. Wochenschr.*, 27, 361 (1914).

<sup>3</sup> *J. Am. Med. Assoc.*, 64, 1489 (1915).

<sup>4</sup> This reagent may be prepared by mixing 10 drops of 37% formaldehyde solution with 10 cc. of sulfuric acid. It should not be used if more than a week or two old.

<sup>5</sup> *Ber.*, 43, 1329 (1910).

<sup>6</sup> *Arch. Pharm.*, 225, 344 and 809 (1887).

alone is precipitated. This method was used by Plugge for the separation of papaverine from narcotine, the two alkaloids having been previously separated from thebaine by precipitation by a concentrated solution of sodium acetate.

The writer has observed that if the precipitate of papaverine ferricyanide, obtained as above described, be collected on a filter, washed with a little water and a small particle of the mass dissolved with stirring in a few drops of sulfuric acid which contains a little formaldehyde solution, a light blue color is produced at once; after a few minutes the color changes to bluish violet (sometimes purplish violet); if the solution be not further disturbed it becomes green at the edges and eventually becomes emerald green; on further standing the color fades to a dirty, brownish yellow or the solution may become nearly colorless. The reaction requires about 30 to 40 minutes for completion. Good results are obtained if about 0.001 g. of the dried papaverine ferricyanide be dissolved in about 0.1 cc. of Marquis' reagent. The reaction does not occur if sulfuric acid which does not contain formaldehyde be used, although a purplish color may be produced. Hexamethylenamine may be used in place of formaldehyde solution, although the initial color is somewhat slow in appearing and may be greenish blue rather than blue. Other reducing agents, such as formic acid, phenylhydrazine hydrochloride, sodium sulfite or sodium thiosulfate, if used in place of formaldehyde, do not give the reaction.

The test was applied to the ferricyanides of several of the alkaloids which form sparingly soluble ferricyanides. The cephaeline and emetine salts gave no colors; the narcotine salt gave a fugitive violet; the strychnine salt gave the well-known "fading purple" reaction. In the last two experiments the presence of formaldehyde did not appear to have any influence in the reaction, as the colors were given by the alkaloidal salts and sulfuric acid without that reagent.

The test was then varied by mixing finely powdered papaverine alkaloid with finely powdered potassium ferricyanide and stirring the mixture with Marquis' reagent. With some variations the succession of colors above noted, blue (greenish blue; see below), violet, green and brownish yellow, was produced, and the deep rose color, characteristic of papaverine with Marquis' reagent, did not appear. In this case it was noted that the initial color was a faintly greenish blue rather than a pure blue and in some instances the emerald green stage was not very pronounced, the violet being succeeded by the brownish yellow color. Potassium ferrocyanide can not be substituted for the ferricyanide in the reaction.

The test (alkaloid + potassium ferricyanide + Marquis' reagent) was then applied to a considerable number of alkaloids or their salts. In most cases either no color was produced or the color was not different

from the control in which no potassium ferricyanide was used. The substances studied were:

Aconitine, apomorphine hydrochloride, atropine sulfate, berberine hydrochloride, beta-eucaine lactate, brucine, caffeine, cephaeline hydrochloride, cinchonidine, cinchonine, cocaine hydrochloride, codeine, colchicine, coniine hydrobromide, dionine, emetine hydrochloride, mixed alkaloids from gelsemium, heroine, homatropine hydrobromide, hydrastine, morphine, narceine, nicotine, novocaine, physostigmine sulfate, pilocarpin hydrochloride, pseudomorphine, pyridine, quinine, sanguinarine nitrate, solanine, sparteine sulfate, strychnine, thebaine, theobromine, and the mixed alkaloids from veratrum.

None of the alkaloids tried except berberine, brucine, colchicine, hydrastine and physostigmine gave reactions differing markedly from the controls. Berberine gave a chocolate brown color, the control being lemon yellow; brucine, an orange-red color with colorless control; colchicine a reddish brown color with yellow control; hydrastine a brownish red color with colorless control; and physostigmine a pale, brownish yellow color with colorless control. No attempt was made to determine whether the color reactions noted with the last named alkaloids had been previously described or whether they were characteristic. In no case was there a duplication or simulation of the reaction with papaverine noted earlier in this paper.

The experiment with papaverine was then varied by substituting various oxidizing agents, such as ammonium vanadate, manganese dioxide, potassium permanganate, selenious acid, etc., for the potassium ferricyanide, in each case a very small quantity of the finely powdered reagent being well mixed with the finely powdered alkaloid and the mixture thoroughly stirred into a few drops of Marquis' reagent. Color reactions were given with a considerable number of the reagents tried, the colors in general being about the same as with potassium ferricyanide, with some individual variations in shade. Reactions were given by ammonium vanadate, cerium oxide, ferric ammonium sulfate, ferric chloride (solid), lead peroxide, manganese dioxide, phosphomolybdic acid, potassium bromate, potassium chlorate, potassium dichromate, potassium iodate, potassium nitrate, selenious acid, silver nitrate, sodium ortho-arsenate and uranium nitrate. Of these, the colors produced by the iron, silver and uranium salts were not very satisfactory. Bromates, chlorates, and nitrates were, in general, found not to give good results, as the oxidation apparently was carried out too rapidly to give a satisfactory play of colors. Ammonium molybdate, ammonium persulfate, magnesium peroxide, phosphotungstic acid or mercuric oxide did not give the reaction. Of the oxidizing reagents tried ammonium vanadate, cerium oxide, potassium permanganate and selenious acid were, perhaps, as satisfactory as any.

One of the most striking reactions observed was obtained by using potassium permanganate as the oxidizer. A very small crystal of the

salt was crushed with a glass rod, about 0.0005 g. of papaverine intimately mixed with the powder and the mixture stirred into about 0.2 cc. of Marquis' reagent. A green color appeared which almost instantly became blue. This deepened into an intense violet-blue which after some time became bluish green, then green and later a dirty brown.

Each of the alkaloids to which the test had been applied, using potassium ferricyanide as the oxidizing agent, was then tested by separately employing ammonium vanadate and potassium permanganate. In no case was there a duplication of the papaverine reaction, although codeine and dionine gave colors which were somewhat confusing. By making comparisons with papaverine, however, distinct differences were observable.

After the work recorded above had been completed the author received from Professor John Uri Lloyd a specimen of an unnamed alkaloid (possibly a mixture of alkaloids) which Professor Lloyd had separated from *sanguinaria*. The author also prepared the alkaloidal material according to the directions of Professor Lloyd from *sanguinaria* extracts sent by him for the purpose. On applying the papaverine test to this alkaloidal separate it was found that with certain oxidizing agents the succession of colors produced was somewhat like that with papaverine. Consequently some confusion might result unless comparison tests be carried out with known papaverine. With sodium ortho-arsenate, manganese dioxide, potassium dichromate and potassium iodate the unnamed alkaloidal substance gave an intense emerald green color which soon showed bluish streaks and faded through green to a dirty, yellowish brown. With the same reagents papaverine gave a momentary greenish blue which passed through deep blue, violet-blue, to green and brownish yellow. However, if selenious acid be used as the oxidizing reagent the initial color produced by the unnamed alkaloidal separate is an intense purplish violet, instead of a fugitive greenish blue which becomes deep blue, as with papaverine. The unnamed alkaloid is further differentiated from papaverine by its solubility in ammonia water, the comparatively ready solubility of its ferricyanide in water, and by the purplish violet, instead of a deep rose color, which it gives with Marquis' reagent.<sup>1</sup>

Although the number of alkaloids to which the newly described test was applied is too small to warrant the conclusion that the reaction described is characteristic for papaverine, yet the considerable number tested without duplication of the color succession leads the author to believe that the reaction may prove of value in the identification of papaverine.

<sup>1</sup> At the request of Professor Lloyd the properties of this unnamed alkaloidal separate from *sanguinaria* are being further investigated and the results will be reported in a separate paper.

### Summary.

1. Very few characteristic color reactions for papaverine are known, the most satisfactory being the deep rose produced by Marquis' reagent.

2. By treating papaverine ferricyanide with Marquis' reagent a blue color is produced which passes through violet and green stages to a dirty, brownish yellow.

3. By treating a mixture of the alkaloid, papaverine, and potassium ferricyanide with Marquis' reagent an essentially identical reaction is produced, although the initial color is generally greenish blue.

4. Many other oxidizing agents may be used in place of potassium ferricyanide, the shades of color produced varying somewhat with the reagent employed.

5. The reaction is best observed by intimately mixing a very small quantity of papaverine with a very small quantity of an oxidizing agent, such as cerium oxide, phosphomolybdic acid or potassium permanganate, and stirring the mixture with a few drops of sulfuric acid containing a little formaldehyde.

6. Of thirty-nine alkaloids tested but one (unnamed alkaloidal separate from sanguinaria) gave colors which in any way simulated the reaction with papaverine.

7. By using selenious acid as the oxidizing agent the unnamed sanguinaria alkaloid (or alkaloids) may be differentiated readily from papaverine.

The thanks of the author are due to Professor John Uri Lloyd for specimens of alkaloids from gelsemium, tobacco, sanguinaria and veratrum kindly furnished by Professor Lloyd for the investigation. Also to Dr. Willis S. Hilpert for verifying some of the reactions.

CHICAGO, ILL.

---

[CONTRIBUTIONS FROM THE SHEFFIELD CHEMICAL LABORATORY OF YALE UNIVERSITY.]

## RESEARCHES ON HYDANTOINS. XXXIV. THE INTERACTION OF HIPPURIC ACID WITH THIOCYANATES.

BY TREAT B. JOHNSON, ARTHUR J. HILL AND BERNARD H. BAILEY.

Received July 19, 1915.

Hippuric acid and dry ammonium thiocyanate interact very smoothly in the presence of acetic anhydride forming 2-thio-3-benzoylhydantoin<sup>1</sup> (I). Potassium thiocyanate likewise interacts to give the same hydantoin, but the yield is about 50% less than that obtained when ammonium thiocyanate is used.<sup>2</sup> Neither ammonium cyanate nor potassium cyanate interact with hippuric acid under similar conditions to give the corresponding 3-benzoylhydantoin (II).

<sup>1</sup> Johnson and Nicolet, *THIS JOURNAL*, **33**, 1973 (1911).

<sup>2</sup> Johnson, *Am. Chem. J.*, **49**, 68 (1913); Johnson and Nicolet, *Ibid.*, **49**, 197 (1913).