MICROCHEMICAL TESTS FOR THE IDENTIFICATION OF ALKALOIDS

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Of the methods available for the identification of minute quantities of alkaloids, the production of microcrystalline derivatives of characteristic form and habit is generally acknowledged to be the most delicate and the most reliable. Colour reactions, while sometimes of great value as confirmatory tests, are not of universal application and suffer from the defect that small traces of impurity may give rise to misleading results. Physiological tests, using living animals, are, on the whole, less sensitive than chemical tests and can only be carried out by persons holding a Home Office licence. Paper chromatography[1] though promising, is as yet largely untried.

Microcrystalline tests have been developed by many workers including Wormley[2], Stephenson[3], Fulton[4,5], White[6], Wagenaar[7], and Oliverio[8], but this development has consisted in the introduction of new reagents rather than in the elaboration of the technique itself. Tests have usually been carried out by placing a drop of the test solution on a microscope slide, adding a drop of the reagent, and examining under low magnification. As this method does not give satisfactory results when dealing with quantities of less than 1 mg., the technique described below has been introduced for the identification of alkaloids when only a few μg. are available.

Microcrystalline Tests

Reagents. Although some 200 different reagents have been described[9,10], only 20 or so are necessary for the identification of the more common alkaloids, although others may be useful for final confirmation. In our opinion, one might well abandon the use of terms such as "Wagner's reagent" or "Mayer's reagent", which, in any case, can only properly be used when referring to a solution of the exact composition described by the original author. Use of the term "Wagner's reagent" for any solution of iodine in potassium iodide can lead to considerable confusion, as the action of this reagent on certain alkaloids depends to a large extent on the iodine/potassium iodide ratio[9,10], We suggest that it is more satisfactory to use the simplest chemical name that will indicate the nature of the reagent.

Details of the reagents in the tests to be described are given in Table I. Some of these are of the usual concentration but others have been modified to meet the special needs of our technique.

Apparatus

Pedestal slides. These are made by cementing small pieces of broken cover-slip to act as distance pieces on opposite sides of the depression of an ordinary cavity slide.

Cover-slips. Circular cover-slips, 0·75 in. in diameter and 0·01 in. in thickness are the most satisfactory. They must be absolutely clean, as the slightest trace of grease causes the drop of liquid to become spherical, thus rendering its examination under the microscope extremely difficult.

"*Micro-rods*". A piece of glass rod, 5 mm. in diameter and 12 cm. long, is heated in the middle and pulled out until its length is about 20 cm. It is then broken at its thinnest point, which should have a diameter of rather less than 1 mm. The narrow ends are ground flat and thoroughly washed to remove glass splinters.

Stand. It is convenient to rest the cover-slip while the test is being made on one end of a short piece of glass tubing, 0·5 in. in diameter, supported in a large rubber bung.

Technique

Each test is carried out with a "microdrop" whose volume is approximately 0·1 cu. mm. The use of so small a drop involves certain practical difficulties, as, if placed on an open slide, it would evaporate so quickly that the reagent would crystallise out and mask the microcrystals of the derivative before they had time to form. On the other hand, complete sealing of the drop in a capillary tube as advocated by Bamford[11], prevents

TABLE I

REAGENTS FOR MICROCHEMICAL TESTS

1.	Gold bromide	5 g. $AuCl_3$ + 5 g. NaBr in 100 ml. of water
2.	Gold bromide/hydrochloric acid	5 g. $AuCl_3$ + 5 g. NaBr in 100 ml. of concentrated hydrochloric acid
3.	Gold chloride	5 per cent. solution
4.	Lead iodide	Adjust 30 per cent. lead acetate solution to *p*H 6 with acetic acid, and saturate with lead iodide
5.	Mercuric chloride	5 per cent. solution
6.	Picric acid	5 per cent. solution
7.	Platinic chloride	5 per cent. solution
8.	Platinic iodide	5 g. of $PtCl_4$ + 25 g. of NaI in 100 ml. of water
9.	Potassium bismuth iodide	5 g. of bismuth subnitrate + 25 g. of KI in 100 ml. of 2 per cent. sulphuric acid
10.	Potassium cadmium iodide	1 g. of CdI_2 + 2 g. of KI in 100 ml. of water
11.	Potassium chromate	5 per cent. solution
12.	Potassium ferrocyanide/hydrochloric acid	Mix equal volumes of 1 per cent. potasium ferrocyanide and 0·5N hydrochloric acid
13.	Potassium iodide	5 per cent. solution
14.	Potassium mercuric iodide	1·5 g. of $HgCl_2$ + 5 g. of KI in 100 ml. of water
15.	Potassium permanganate	2 g. of $KMnO_4$ in 100 ml. of water + 5 drops syrupy phosphoric acid
16.	Potassium tri-iodide (1)	2 g. of I_2 + 4 g. of KI in 100 ml. of water
17.	Potassium tri-iodide (2)	0·1 g. of I_2 + 0·2 g. of KI in 100 ml. of water
18.	Potassium tri-iodide (3)	1 g. of I_2 + 50 g. of KI in 100 ml. of water
19.	Sodium carbonate	5 per cent. solution
20.	Sodium phosphate	5 per cent. solution of Na_2HPO_4
21.	Zinc chloride	5 per cent. solution

any evaporation, which is essential when dealing with dilute solutions, or with salts such as chromates which have appreciable solubility. The use of a cover-slip not only inhibits evaporation, but has the added disadvantage of distorting and rendering unrecognisable the crystals that form in the thin film between cover-slip and slide.

In order to overcome these difficulties a hanging drop technique has been introduced. The microdrop hangs from a cover-slip held away from a cavity slide by distance pieces. Slow evaporation can thus take

place but may be stopped at any instant by "ringing" the cover-slip. The material to be tested should be dissolved in 1 per cent. acetic acid (or in 1 per cent. hydrochloric acid if insoluble in the former). The surface of this solution is then touched with a micro-rod, and the adhering drop of liquid transferred to a cover-slip placed on the stand. A similar drop of reagent is now added by means of another micro-rod, and the drop stirred.

TABLE II

RESULTS OF MICROCHEMICAL TESTS

Alkaloid	Reagent	Crystals	Sensitivity µg.
Aconitine	(i) Sodium carbonate	Rosettes	0·25
	(ii) Potassium permanganate	Dense rosettes of rods	0·25
Apomorphine	(i) Potassium bismuth iodide	Rosettes of needles (ON)	0·05
	(ii) Gold bromide	Serrated crosses	0·05
Arecoline	(i) Potassium bismuth iodide	Red rhomboids	0·025
	(ii) Picric acid	Small dense rosettes	0·25
Atropine	(i) Potassium tri-iodide (3)	Rhomboids and wedge shaped crystals	0·05
	(ii) Picric acid	Bunches of plates	0·25
Brucine	(i) Platinum chloride	Needles	0·05
	(ii) Potassium chromate	Bunches of rods	0·025
Caffeine	(i) Mercuric chloride	Long needles	0·05
	(ii) Gold chloride	Small rods	0·05
Cinchonidine	(i) Sodium carbonate	Small rosettes	0·1
	(ii) Platinum chloride	Small rods	0·025
Cinchonine	(i) Sodium carbonate	Rosettes	0·025
	(ii) Platinum iodide	Curved needles (ON)	0·05
Cocaine	(i) Lead iodide	Feathery rosettes	0·025
	(ii) Gold chloride	Serrated needles	0·025
Codeine	(i) Potassium cadmium iodide	Rosettes of rods	0·05
	(ii) Potassium tri-iodide (1)	Feathery rosettes (ON)	0·1
Cotarnine	(i) Mercuric chloride	Long needles	0·05
	(ii) Platinum chloride	Rods	0·025
Ephedrine	(i) Potassium bismuth iodide	Branching needles	0·1
	(ii) Platinum chloride	Curving oily needles	0·1
Diamorphine	(i) Mercuric chloride	Fine branching needles	0·1
	(ii) Platinum chloride	Rosettes	0·5
Homatropine	(i) Potassium bismuth iodide	Bunches of overlapping plates	0·05
	(ii) Potassium tri-iodide (3)	Serrated plates	0·05
Hydrastine	(i) Sodium carbonate	Rosettes	0·5
	(ii) Potassium ferrocyanide/hydrochloric acid	Dense rosettes	0·5
Hydrastinine	(i) Potassium permanganate	Serrated blades	0·05
	(ii) Mercuric chloride	Branching needles	0·05
Hyoscyamine	(i) Potassium tri-iodide (3)	Small plates	0·05
	(ii) Picric acid	Rosettes of needles	0·25
Morphine	(i) Potassium cadmium iodide	Needles	0·025
	(ii) Potassium tri-iodide (1)	Orange plates	0·1
Narceine	(i) Potassium tri-iodide (2)	Tufts of fine needles	0·025
	(ii) Lead iodide	Thread-like needles	0·05
Narcotine	(i) Sodium carbonate	Small rosettes	0·05
	(ii) Potassium chromate	Feathery rosettes	0·05
Nicotine	(i) Mercuric chloride	Rosettes of blades	0·05
	(ii) Picric acid	Branching needles	0·025
Papaverine	(i) Zinc chloride	Bunches of plates	0·025
	(ii) Potassium cadmium iodide	Rosettes of blades	0·05
Physostigmine	(i) Gold bromide/hydrochloric acid	Dark serrated needles	0·025
	(ii) Lead iodide	Serrated blades	0·25
Pilocarpine	(i) Platinum chloride	Plates in clusters	0·05
	(ii) Gold bromide	Feathery rosettes	0·05
Quinidine	(i) Potassium iodide	Irregular crystals often triangular	0·25
	(ii) Sodium carbonate	Dense rosettes (ON)	0·1
Quinine	(i) Sodium phosphate	Needles	0·1
	(ii) Platinum iodide	Curved serrated needles (ON)	0·05
Scopolamine	(i) Potassium bismuth iodide	Rosettes of plates (ON)	0·05
	(ii) Gold bromide	Serrated yellow plates	0·05
Sparteine	(i) Gold chloride	Small irregular blades	0·025
	(ii) Potassium cadmium iodide	Long plates	0·05
Strychnine	(i) Potassium mercuric iodide	Wedge shaped crystals sometimes curved	0·001
	(ii) Gold chloride	Feathery rosettes	0·01
Thebaine	(i) Sodium carbonate	Rosettes	0·05
	(ii) Platinum chloride	Rosettes of small plates	0·1

ON (overnight) indicates that the crystals do not usually form until the following day.

The distance pieces of a pedestal slide are moistened with a little gum arabic solution, and the cover-slip picked up by inverting the slide over it and pressing the distance pieces against its edges. The whole is now re-inverted and examined under the low power of the microscope.

If an appreciable precipitate, either crystalline or amorphous can be seen, the slide is sealed at once, by "ringing" the cover-slip with gum arabic solution, thus inhibiting further evaporation. If, however, no precipitate has been formed, sealing is deferred until the drop has been sufficiently concentrated by evaporation for precipitation to begin, care being taken not to delay until the reagent begins to crystallise. If no crystals have been formed when the slide is sealed it is examined at intervals for the next twenty-four hours, as some substances are very slow to crystallise. Once crystallisation is complete, the shape of the crystals is noted and photographs may be taken for purposes of record, as, although many crystal formations are permanent, others disintegrate fairly quickly.

Results. In Table II two tests are given for each of a number of the more common alkaloids. Some of these tests are well known, and are given in the "Methods of Analysis" of the Association of Official Agricultural Chemists[12] and elsewhere, but others have been introduced as most suitable for use on the microscale. Figures 1 to 6 illustrate some typical crystals, the quantity used being indicated. It cannot be emphasised too strongly, however, that descriptions and photographs can only enable a provisional identification to be made. Final identification can only be made by comparing the crystals obtained from the test solution with those from the same reagent and a control solution of comparable strength made from a known sample of the alkaloid in question.

Colour Tests

Schemes for the identification of alkaloids by means of colour reactions have been worked out by Bamford[12] and Jackson[13]. This method has also been thoroughly investigated by Farmilo, Levi, Oestreicher and Ross[14]. In spite of the claims made for it, we do not consider this method as delicate or as reliable as the microcrystalline tests. Nevertheless, colour tests are sometimes of great value, and a method is described below for using them, employing microdrops, to identify quantities of the order of 0·1 μg. In some cases the original tests have had to be modified to give satisfactory results at this level. Thus the usual method for many of these tests is to dissolve the reagent in concentrated sulphuric acid, and to add a drop of this solution to a particle of the unknown substance. We find, however, that by adding a microdrop of an aqueous solution of the reagent to a microdrop of the test solution, evaporating to dryness, and moistening the residue with a rod dipped in concentrated sulphuric acid, the test may be made a good deal more sensitive, although the colours observed are not necessarily the same.

The tests are carried out on pieces of opal glass the size of a microscope slide. As the amount of solid residue left after evaporation is so small as to be almost invisible it is convenient to rule an interrupted line, having a number of gaps about $\frac{1}{8}$ in. wide, down the centre of the glass. If the

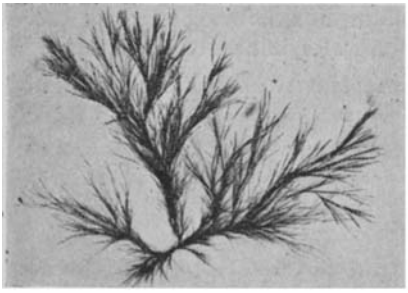

FIG. 1. Codeine (0·025 μg.) with potassium tri-iodide (I). × 50.

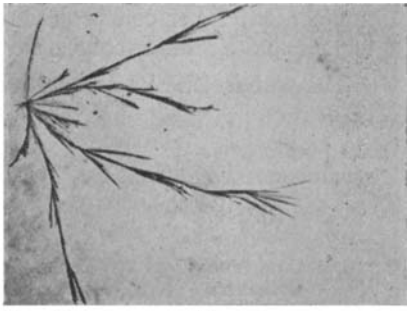

FIG. 2. Apomorphine (0·1 μg.) with potassium bismuth iodide × 50.

FIG. 3. Morphine (0·1 μg.) with potassium cadmium iodide × 50.

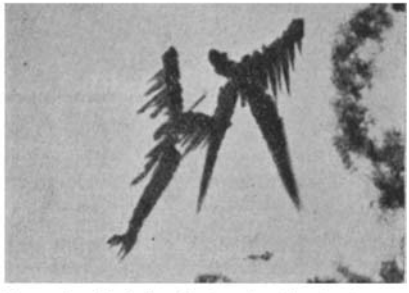

FIG. 4. Quinine (0·1 μg.) with platinum iodide × 250.

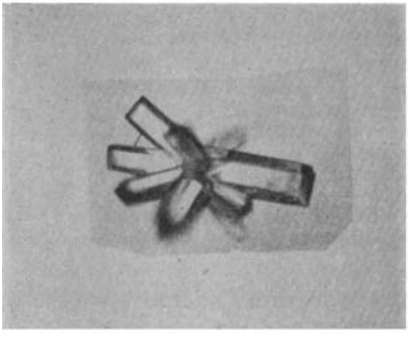

FIG. 5. Thebaine (0·1 μg.) with sodium carbonate × 200.

FIG. 6. Arecoline (0·025 μg.) with potassium bismuth iodide × 100.

drop is evaporated in the centre of one of these gaps, no difficulty will be experienced in adding the reagent to the residue.

For the vanadate test (Mandelin) a microdrop of a saturated aqueous solution of ammonium vanadate is added to a microdrop of the test solution, and the residue after evaporation is treated with a microdrop of concentrated sulphuric acid. The selenium dioxide test (Mecke) and

the ammonium molybdate test (Froehde) are carried out in the same manner, a 0·5 per cent. solution of selenium dioxide being used for the former and a similar solution of ammonium molybdate for the latter.

The colours obtained, together with the sensitivity of the test, are given below.

Ammonium Vanadate Test

Alkaloid	*Colour*	*Sensitivity μg.*
Cotarnine	Orange——brown	0·05
Ephedrine	Brick red, quickly fading	0·5
Hydrastine	Reddish brown—red	0·1
Hydrastinine	Orange——pale green	0·05
Narceine	Dark brown	0·1
Narcotine	Pink——brown, fading	0·2
Papaverine	Dirty green	0·2
Strychnine	Blue——purple——red	0·05
Thebaine	Reddish brown	0·1

Selenium Dioxide Test

Apomorphine	Blue-black——green——brown	0·1
Codeine	Blue-green——yellow-green——brown	0·5
Cotarnine	Yellow-brown	0·5
Diamorphine	Blue-green——olive-green——brown	0·5
Hydrastine	Pale-green——brown	0·25
Hydrastinine	Yellow	0·25
Morphine	Blue-green——grey-green	0·1
Narceine	Bright-green——grey——orange	0·25
Narcotine	Green——orange	0·25
Papaverine	Grey——grey-green, fading	0·25
Thebaine	Green——brown——orange	0·25

Ammonium Molybdate Test

Apomorphine	Deep green——blue——green	0·1
Codeine	Blue, slowly fading	0·1
Cotarnine	Pale violet——green	0·25
Diamorphine	Red violet——blue——light green	0·05
Hydrastine	Grey-green——blue——pale green	0·05
Hydrastinine	Yellow——green, fading	0·25
Morphine	Violet——blue——light green	0·05
Narceine	Brown——grey——blue——green	0·05
Narcotine	Brown——green——blue——pale green	0·05
Thebaine	Greenish brown——red brown	0·1

Vitali's test is carried out by treating the residue from a microdrop of the test solution with fuming nitric acid, evaporating to dryness and moistening the residue with a drop of a freshly prepared ethanolic solution of potassium hydroxide. Atropine, hyoscyamine, and scopolamine all give a deep violet colour, the sensitivity being about 0·01 μg.

The formaldehyde-sulphuric acid reagent (Marquis) is made by adding 1 drop of 40 per cent. formaldehyde to 1 ml. of concentrated sulphuric acid. A microdrop of the test solution is evaporated, and the residue rubbed with a rod dipped in the reagent. The colours observed are given below.

Formaldehyde-sulphuric Test

Alkaloid	*Colour*	*Sensitivity* μg.
Apomorphine	Purple——black	0·05
Codeine	Violet	0·05
Diamorphine	Violet	0·05
Morphine	Violet	0·05
Narceine	Brown——deep brown——green	0·05
Narcotine	Bluish violet, quickly fading	0·1
Thebaine	Red——orange	0·05

Several other colour tests may be carried out in a similar manner, but we have found them of little practical value when dealing with quantities of less than 1 μg.

Discussion

It will be seen that there is wide variation in the sensitivity of the microcrystalline tests for different alkaloids. Thus while 0·001 μg. of strychnine will give recognisable crystals, we have been unable to find any test for hydrastine that is reliable when less than 0·5 μg. is used. Nevertheless, use of this technique should enable 10 μg. of any common alkaloid to be identified without difficulty, especially if tests with the reagents most likely to give crystals, such as platinum chloride or mercuric chloride, are carried out first. Three points, however, must be borne in mind: (1) There are many more alkaloidal substances, both natural and artificial, than those included in Table II. (2) The tests given in this table are only those that we have found to be the most satisfactory. The list is not intended to be exhaustive. Strychnine, for example, will give crystals with a number of these reagents. (3) No single tests is specific. For definite identification at least two positive microcrystalline tests must be made. If a colour tests is available, it may be used to afford additional verification. As the detection of alkaloids is frequently of forensic significance, absolute certainty in identifications is essential. This technique, in which 1 drop (0·05 ml.) provides material for no less than 500 different tests, is exceptionally valuable in that it allows additional confirmatory tests to be made, and doubtful tests to be repeated.

Summary

1. The available methods for the identification of minute quantities of alkaloids are discussed.

2. A technique is described for identifying alkaloids by microcrystalline tests, the quantity needed for the test being in the range 1 μg. to 0·01 μg.

3. Descriptions are given of the crystals obtained from 30 different alkaloids with various reagents using this technique. Details of the reagents used are also given.

4. Modifications of certain colour tests are described.

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