MICROCHEMICAL IDENTIFICATION OF SOME LESS COMMON ALKALOIDS

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During recent years new techniques have been developed for the identification of alkaloids and older techniques have been modified[1,2], but the tendency has been to describe each new development as applied to the same group of twenty or thirty well known alkaloids. The result is that of the thousands of alkaloids now known, micro tests suitable for medico-legal work have been described for fewer than a hundred. The toxicological chemist must be in a position to give positive identification to any alkaloidal substance that he isolates, and not merely to a selected few of classical forensic interest. This paper describes crystal and colour tests for 40 alkaloids which, although less well known, are all substances that may either be obtained commercially or extracted with comparative ease from common plants; most of these compounds are referred to by Henry[3] or Manske and Holmes[4]. Of the more recently discovered ones, aquaticine was isolated from *Senecio aquaticus* by Evans and Rees Evans[5], and demecolcine (desacetylmethylcolchicine) from *Colchicum autumnale* by Santavy and Reichstein[6].

Experimental Procedure

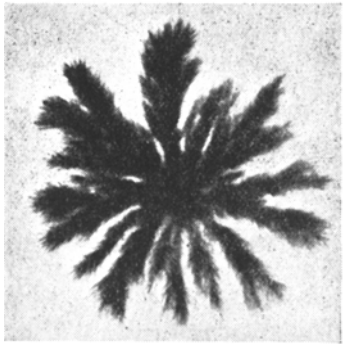
Microcrystalline tests:—The hanging-microdrop technique described by Clarke and Williams[7] was employed. The alkaloids were dissolved in 1 per cent acetic acid or 1 per cent hydrochloric acid except in the following cases: piperine was dissolved in ethanol, theobromine in a mixture of 1 volume of concentrated hydrochloric acid and 2 volumes of water, and reserpine in a mixture of 50 volumes of ethanol, 50 volumes of water, and 1 volume of glacial acetic acid.

TABLE I

Reagents

Ammonium thiocyanate ..	5 per cent solution
Di-sodium methyl arsonate ..	5 per cent solution
Gold cyanide	Dissolve 5g. gold chloride in 100 ml. water, and add solid sodium cyanide till the precipitate redissolves
Picrolonic acid	Saturated solution
Platinum bromide	5 g. platinum chloride and 10 g. sodium bromide in 100 ml. water
Potassium cyanide	5 per cent solution
Styphnic acid	5 per cent solution
Trinitrobenzoic acid	Saturated solution

In spite of every effort to restrict the number of reagents employed, it was found necessary to use several solutions in addition to those previously described[7,8]. Details of these are given in Table I. In the course of this work many other reagents have been tested, but have been discarded as of no value.



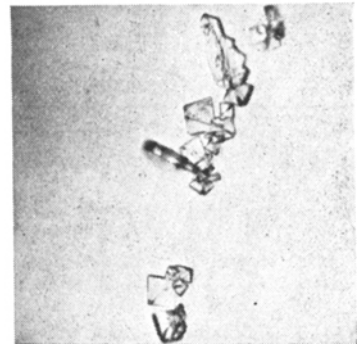
A. Ergometrine (1·0 μg.) with trinitrobenzoic acid.

B. Berberine (0·5 μg.) with picric acid.

C. Cytisine (0·25 μg.) with potassium mercuric iodide.

D. Demecolcine (0·5 μg.) with gold chloride.

E. Reserpine (0·5 μg.) with ammonium thiocyanate.



F. Dimethyltubocurarine (0·25 μg.) with potassium iodide.

FIG. 1.

TABLE II

Alkaloid	Reagent	Crystals	Sensitivity μg.
Agmatine	Picric acid	Small irregular rhomboids	0·025
	Platinum iodide	Rods and rhomboids	0·025
α-Allocryptopine.	Gold cyanide	Feathery rosettes*	0·25
	Potassium iodide	Dense rosettes*	0.5
Aquaticine	Potassium bismuth iodide	Small branching needles	0·025
	Sodium carbonate	Plates or serrated blades	0·1
Benzoyl ecgonine	Gold chloride	Long plates and needles	0.05
	Potassium permanganate	Branching needles	0·25
Berberine	Picric acid	Curved needles	0.025
	Gold cyanide	Feathery rosettes	0·025
Betaine	Platinum iodide	Rectangular plates	0·5
	Gold chloride	Rods and plates	1·0
Bicuculline	Sodium carbonate	Dense rosettes*	0·05
	Potassium chromate	Dense rosettes*	0·25
Boldine	Mercuric chloride	Dense rosettes	0·25
	Gold cyanide	Long needles*	0·5
Chelidonine	Sodium carbonate	Serrated plates	0·1
	Zinc chloride	Irregular prisms	0·25
Coniine	Potassium bismuth iodide	Rhomboids	0·025
	Platinum iodide	Plates	0·25
Corynanthine	Mercuric chloride	Bunches of small plates	0·25
	Potassium iodide	Plates and dense rosettes	0·5
Cryptopine	Picric acid	Rosettes of needles	0·1
	Zinc chloride	Rosettes of very small needles	0·1
Cytisine	Potassium tri-iodide (1)	Short rods	0·025
	Potassium mercuric iodide	Fans of needles	0·1
Demecolcine	Gold chloride	Feathery rosettes	0·1
	Platinum chloride	Dense rosettes	0·5
Dimethyl tubocurarine	Potassium chromate	Bunches of irregular plates	0·025
	Potassium iodide	Plates	0·1
Ecgonine	Potassium bismuth iodide	Hexagons*	0·1
	Platinum iodide	Small rectangular plates*	0·25
Ergometrine	Picric acid	Clumps or fans of rods	0·5
	Trinitrobenzoic acid	Dendrites	0·5
Ethyl hydrocupreine	Ammonium thiocyanate	Needles	0·25
	Sodium phosphate	Rosettes of rods	1·0
Ethyl papaverine	Zinc chloride	Bunches of plates	0·05
	Potassium iodide	Rosettes of curved blades	0·05
Galegine	Picric acid	Curved needles	0·05
	Styphnic acid	Stout needles	0·05
Gramine	Gold bromide	Rosettes of feathery needles	0·025
	Potassium bismuth iodide	Prisms, often rectangular*	0·025
Harmine	Gold chloride	Rods	0·025
	Potassium iodide	Needles	0·025
Hordenine	Potassium bismuth iodide	Needles or rhomboids	0·05
	Gold bromide	Long needles*	0·1
Hydroquinidine	Gold bromide/hydrochloric acid	Branching needles	0·025
	Gold chloride	Branching needles	0·025
Hydroquinine	Platinum bromide	Bunches of plates	0·05
	Mercuric chloride	Large needles	0·025
α-Lobeline	Sodium carbonate	Rosettes	0·025
	Potassium cyanide	Rosettes	0·025
Lupanine	Potassium mercuric iodide	Bunches of feathery needles*	0·025
	Gold bromide	Bunches of plates	0·05
Lycorine	Picric acid	Plates	0·5
	Potassium cyanide	Stout needles	0·5
Mescaline	Potassium bismuth iodide	Dense rosettes	0·025
	Styphnic acid	Small needles	0·025
Methyl ergometrine	Picric acid	Rosettes or fans of needles	0·5
	Trinitrobenzoic acid	Feathery rosettes	0·5
Piperine	Sodium carbonate	Long plates	0·25
	Trinitrobenzoic acid	Small needles	0·1
Protopine	Picric acid	Minute dense rosettes	0·025
	Picrolonic acid	Rosettes of hair like needles	0·025
Protoveratrine 'A'	Di-sodium methyl arsonate	Dense rosettes	0·5
	Sodium carbonate	Oily rosettes	0·25
Protoveratrine 'B'	Di-sodium methyl arsonate	Dense rosettes	0·5
	Picric acid	Oily dendrites*	1·0
Reserpine	Potassium cyanide	Small rosettes*	0·1
	Ammonium thiocyanate	Plates*	0·1
Theobromine	Gold bromide	Hair like needles†	0·025
	Platinum iodide	Small rods and plates	0·1
Theophylline	Gold bromide/hydrochloric acid	Needles†	0·25
	Mercuric chloride	Sheaves of broad needles	0·25
Trigonelline	Gold chloride	Fine needles	0·25
	Potassium tri-iodide (1)	Plates	0·5
Tropine	Gold bromide	Rhomboids	0·025
	Picric acid	Needles	0·025
Tubocurarine	Zinc chloride	Rosettes of small plates*	0·5
	Sodium phosphate	Very small needles*	0·5
Yohimbine	Sodium carbonate	Rosettes of rods	0·05
	Potassium cyanide	Rosettes of rods	0·05

*Crystals form very slowly †Crystals not stable

TABLE III

COLOUR TESTS

Alkaloid	Colour	Sensitivity μg.
Formaldehyde-sulphuric acid test 'Marquis'		
α-Allocryptopine	Mauve	0·1
Berberine	Yellow-green	0·25
Bicuculline	Orange	1·0
Boldine	Green—purple—green	0·1
Chelidonine	Faint green	0·5
Corynanthine	Brown—purple-brown	0·5
Cryptopine	Blue—green	0·1
Demecolcine	Yellow	0·25
Ethyl papaverine	Faint brown	1·0
Ergometrine	Brown	0·25
Gramine	Grey-brown	0·25
Harmine	Orange—grey	0·025
Hordenine	Brown—green	0·5
Lobeline	Red-violet—brown	0·1
Mescaline	Orange	0·1
Methyl ergometrine	Brown	0·25
Piperine	Orange—brown	0·5
Protopine	Blue—green	0·025
Reserpine	Blue—grey-green—brown	0·1
Ammonium vanadate test		
α-Allocryptopine	Purple—yellow	0·25
Berberine	Blue-green—mauve	0·025
Bicuculline	Red	0·25
Boldine	Green—brown	0·25
Chelidonine	Yellow—green	0·5
Corynanthine	Blue—green	0·25
Cryptopine	Violet—blue—green	0·25
Demecolcine	Purple—yellow	0·25
Ethyl papaverine	Green	1·0
Ergometrine	Grey-green—grey-purple	0·5
Gramine	Violet—yellow-green	0·05
Harmine	Blue—green	0·025
Lobeline	Grey	0·5
Mescaline	Orange	0·25
Methyl ergometrine	Grey-green—grey-purple	0·5
Piperine	Red-brown—green	0·25
Protopine	Violet—blue—green	0·05
Reserpine	Blue—brown-purple	0·1
Yohimbine	Blue—green	0·1
Ammonium molybdate test		
α-Allocryptopine	Green—violet—yellow-green	0·1
Berberine	Brown—grey	0·025
Bicuculline	Blue-green—yellow-green	0·25
Boldine	Blue—blue-green	0·1
Chelidonine	Green—blue—green	0·1
Corynanthine	Blue—green	0·1
Cryptopine	Green—violet—green	0·1
Demecolcine	Green—yellow	0·25
Dimethyl tubocurarine	Blue—green	0·5
Ethyl papaverine	Green—blue—green	0·1
Ergometrine	Green—brown	0·25
Galegine	Blue	1·0
Gramine	Blue—yellow-green	0·025
Harmine	Yellow—grey	0·025
Hordenine	Blue—green—yellow	0·25
Lobeline	Grey—pink	1·0
Lupanine	Faint blue	1·0
Lycorine	Green—blue—yellow	0·25
Mescaline	Green-blue	0·05
Methyl ergometrine	Green—brown	0·25
Piperine	Red-brown	0·25
Protopine	Green—violet—green	0·025
Reserpine	Blue	0·1
Tubocurarine	Blue-green	0·5
Yohimbine	Blue—green	0·025
Selenium dioxide test		
α-Allocryptopine	Violet—red-brown	0·1
Berberine	Brown—purple	0·05
Bicuculline	Yellow—brown	1·0
Boldine	Green—brown	0·25
Chelidonine	Yellow—brown—orange	0·25
Corynanthine	Blue-green—green	0·25

TABLE III—*continued*

Alkaloid	Colour	Sensitivity μg.
Selenium dioxide test—*continued*		
Cryptopine	Violet	0·1
Demecolcine	Yellow	0·25
Dimethyl tubocurarine	Brown—orange	0·5
Ethyl papaverine	Grey	0·1
Ergometrine	Green—brown	0·25
Galegine	Orange	1·0
Gramine	Grey-green—grey-brown	0·25
Harmine	Green—yellow	0·05
Lobeline	Yellow-brown	1·0
Lycorine	Brown	0·5
Mescaline	Yellow-brown	0·1
Methyl ergometrine	Green—brown	0·25
Piperine	Brown—green	0·1
Protopine	Purple—brown	0·05
Reserpine	Olive	0·1
Tropine	Greenish-brown—purple-brown	0·5
Tubocurarine	Brown—orange	0·5
Yohimbine	Blue—green	0·1
Vitali's test		
α-Allocryptopine	Yellow/brown/brown	0·5
Berberine	Brown/brown/violet	0·025
Bicuculline	Yellow/brown/light-brown	0·5
Boldine	Brown-orange/brown/dark-brown	0·1
Chelidonine	Yellow/brown/brown	0·5
Corynanthine	Yellow/yellow/red-violet	0·1
Cryptopine	Yellow/brown/brown	0·5
Demecolcine	Yellow—purple/yellow/red	0·25
Ethyl papaverine	— /yellow-brown/brown	1·0
Ergometrine	Yellow-brown/yellow-brown/purple-brown	0·1
Gramine	Yellow/orange/red-brown	0·1
Harmine	Green/grey/orange—pink	0·025
Hordenine	— / — /orange	0·1
Lycorine	Yellow/orange/orange	0·5
Mescaline	Violet/brown/brown	0·25
Methyl ergometrine	Yellow-brown/yellow-brown/purple-brown	0·1
Piperine	Yellow/yellow/ —	1·0
Protopine	Yellow/brown/dark-brown	0·5
Reserpine	Orange/orange/brown	1·0
Yohimbine	Yellow/yellow/violet	0·1

The results obtained are shown in Table II. In order to save space, only two tests have been given for each alkaloid. It must, however, be realised that many alkaloids form crystalline derivatives with a number of reagents. Unless this fact is borne in mind, some confusion may arise in applying these tests. Thus both tubocurarine and dimethyl-tubocurarine form small plates with zinc chloride solution; but only the latter forms crystals with potassium chromate and potassium iodide. Some typical crystals are shown in Figure 1.

Colour tests:—These are carried out with microdrops on opal glass as previously described[7]. The results obtained are given above in Table III. In the case of Vitali's test the colours shown are those given on addition of the nitric acid, after evaporation, and on addition of the ethanolic potash respectively.

In both crystal and colour tests, final identification rests on the comparison of the results obtained from the test material with those obtained from a control solution made from a known sample of the suspected alkaloid. If a number of controls of varying dilution are employed, the objection raised by Pedley[9] that the crystals vary with concentration is largely overcome. In addition, a rough approximation of the strength of the test solution may be obtained.

Discussion

Although the classical crystal and colour tests are being abandoned in favour of physicochemical methods such as paper chromatography and ultra-violet spectrophotometry, these latter techniques are really more suited to preliminary screening than to positive identification. Thus if there is no peak at 287 mμ[10] morphine cannot be present; but a positive result does not mean that it must necessarily be so. Similarly an R_F value of 0·25[11] indicates that strychnine is possibly present, but cannot be considered as proof of its presence without confirmatory evidence. As the microcrystal and colour tests described above may easily be applied to substances eluted from paper chromatograms[12], this method forms a convenient means of providing such additional evidence.

All the tests described above were carried out with pure alkaloids but the technique has been used on numerous occasions to identify substances isolated from animal or plant material by a modified Stas-Otto process.

Summary

Crystal and colour tests are described for the identification of 40 of the less common alkaloids.

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