THE IDENTIFICATION OF LOCAL ANÆSTHETIC DRUGS OF THE BENZOIC ESTER GROUP

By E. Hucknall and G. E. Turfitt

From the Metropolitan Police Laboratory

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The property of paralysing sensory nerve endings and inducing local anæsthesia is not confined to any one specific chemical structure. Indeed, from a chemical standpoint it is convenient to arrange local anæsthetics in three groups: (1) consisting mainly of hydroxy compounds (e.g. phenol, menthol) used largely for topical anæsthesia, (2) the benzoic ester group, and (3) various miscellaneous compounds of synthetic origin, and of widely differing chemical structure (e.g., cinchocaine, phenacaine).

By far the largest, and clinically the most important of these groups is that composed of the benzoic esters. Following the demonstration by Koller in 1884 that cocaine, methylecgonine benzoate, is an effective local anæsthetic for ophthalmic purposes, a wide range of benzoic esters has been synthesised and investigated as "cocaine substitutes." Whilst these are, for the most part, less toxic than cocaine itself, and are devoid of the latter's addiction liability, the relative toxicities depend largely upon the manner and rate of administration. At the present time, eleven benzoic ester local anæsthetics are in common use in this country (Table I), and with a single exception (butyl-*p*-aminobenzoate) are characterised by names ending in "-caine." This terminology, although admittedly useful in drawing attention to the local anæsthetic nature of a particular drug, has unfortunately been responsible on occasion for confusion during dispensing and use. Fatal poisonings with these drugs are usually the result of accident due to such confusion, to over-dosage, to intravenous instead of subcutaneous injection, or to individual hypersensitivity; suicidal poisonings are relatively rare.

Information as to the metabolic fate of these drugs is incomplete. Detoxification occurs largely in the liver with hydrolysis of the ester linkage, but there is little doubt that other tissues also play some part in the destruction of the drugs *in vivo*. In the case of cocaine, hydrolysis yields benzoic acid and ecgonine; elimination is relatively slow and the hydrolysis products, together with a proportion of unchanged cocaine, may be excreted *via* the kidneys over a period of several days. With the other local anæsthetics breakdown is, in general, a rapid process, and elimination as acetylated *p*-aminobenzoic acid is usually complete within a few hours. For these reasons, detection of unchanged drugs other than cocaine in cases of fatal poisoning is unusual, except where considerable doses have been administered, and attempts at recovery from cadaveric material after an interval of days are likely to be unsuccessful. Particularly important, however, is the examination of the liver, kidneys and urine, since, although insufficient unchanged drug may be obtained for purposes of identification, products of hydrolysis such as ecgonine and *p*-aminobenzoic acid may be recoverable.

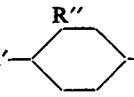
In view of the accidental character of most cases of local anæsthetic poisoning, the toxicologist's problem is seldom the identification of a completely unknown poison, but is usually the confirmation of the presence of a particular local anæsthetic drug. The most successful of the numerous methods hitherto described for differentiating and identifying the drugs of this group is probably that of Fischer[1], who utilises the crystalline forms and melting-points of derivatives formed with the reagents trinitroresorcinol, trinitrobenzoic acid, picric acid and platinic chloride. There appears, however, to be no recorded comprehensive scheme whereby, on a micro scale, an unknown isolated residue may be characterised as a benzoic ester local anæsthetic, and subsequently completely identified.

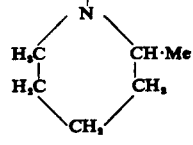
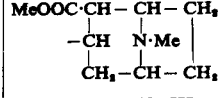
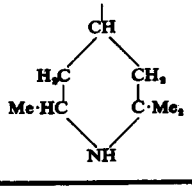
In the present investigation, dealing with the benzoic ester anæsthetics listed in Table I, a residue isolated either from biological material or from a medicinal preparation is examined in a series of separate stages: (1) purification of the crude residue, (2) behaviour with " alkaloidal reagents," (3) demonstration of benzoic ester structure, (4) simple crystal and colour reactions, (5) conclusive identification by micro mixed melting-point determination. It will be evident that the requirements of a particular problem may frequently be met without the inclusion of all these stages, subject to the use, whenever quantity permits, of the mixed melting-point as the final criterion of identity. The tests described are simple techniques, requiring a minimum of time, and employing only readily available reagents and apparatus. With quantities of the order of 1 mg., the scheme provides for the characterisation of an unknown drug as a benzoic ester local anæsthetic, and for its ultimate identification.

Isolation of local anæsthetic drugs. The basic character of the local anæsthetics of the benzoic ester group is such that, in general, they may be extracted with chloroform from solutions alkaline with sodium hydroxide, and thus in the Stas-Otto process are largely obtained in the non-phenolic alkaloid group. At the same time, hydrolysis may occur under the influence of the sodium hydroxide, and the yield of drug may in this way be appreciably reduced. Noteworthy exceptions are the structurally similar benzocaine and butyl *p*-aminobenzoate, which are extracted by ether from aqueous acid solutions, and are, therefore, obtained in the acid-ether extract during the normal Stas-Otto process. In the case of butyl *p*-aminobenzoate, hydrochloric acid is preferable to sulphuric acid for the preparation of the aqueous acid solution owing to the relatively insoluble nature of the sulphate of this drug. The phenolic drug orthocaine undergoes progressive decomposition in the presence of alkali, and dark-coloured residues are obtained in both the non-phenolic and phenolic alkaloid groups following the Stas-Otto procedure. This drug is best extracted with ether from aqueous solutions acidified with tartaric acid.

Purification of crude residues. When isolated from biological sources the drugs are invariably contaminated with brownish, often gummy, extractives. Since ether solutions of the free bases deposit the white hydrochlorides on treating with a stream of dry hydrogen chloride, this

TABLE I

LOCAL ANÆSTHETIC DRUGS. (R′—  —COOR)

	R	R′	R″	Mp. (°C) of hydrochloride
AMETHOCAINE HYDROCHLORIDE B.P. ... Anethaine Butethanol Decicaine Pantocaine Tetracaine	$-CH_2\cdot CH_2\cdot NMe_2$	$C_4H_9\cdot NH-$	H—	149
BUTACAINE SULPHATE B.P. ... Butyn	$-CH_2\cdot CH_2\cdot CH_2\cdot NBu_2$	NH_2-	H—	151
PROCAINE HYDROCHLORIDE B.P. Ethocaine Kerocaine Novocaine Planocaine Sericaine	$-CH_2\cdot CH_2\cdot NEt_2$	NH_2-	H—	155
AMYDRICAINE HYDROCHLORIDE B.P.C. ... Alypin	$-C\,Et\,(CH_2NMe_2)_2$	H—	H—	169
METYCAINE (HYDROCHLORIDE) ...	$-CH_2\cdot CH_2\cdot CH_2$ 	H—	H—	175
AMYLOCAINE HYDROCHLORIDE B.P.C. ... Stovaine	$-C\,(Me)\,(Et)\cdot CH_2\cdot NMe_2$	H—	H—	178
COCAINE B.P.		H—	H—	186*
BUTYL *p*-AMINOBENZOATE B.P. ... Butesin Butoform Scuroforme	$-CH_2\cdot CH_2\cdot CH_2\cdot CH_3$	NH_2-	H—	187
BENZOCAINE B.P. .... Anaesthesin	$-CH_2\cdot CH_3$	NH_2-	H—	195
ORTHOCAINE B.P.... ... ... New Orthoform	$-CH_3$	HO—	NH_2-	225 d.
BENZAMINE HYDROCHLORIDE B.P.C. ... Betacaine HCl... ... ... Betaeucaine HCl Eucaine HCl		H—	H—	268 d.

* The melting point of cocaine is variously reported in the literature to be between 182° and 202°C., the variation being attributable (Smith[8]) to the rate and conditions of heating.

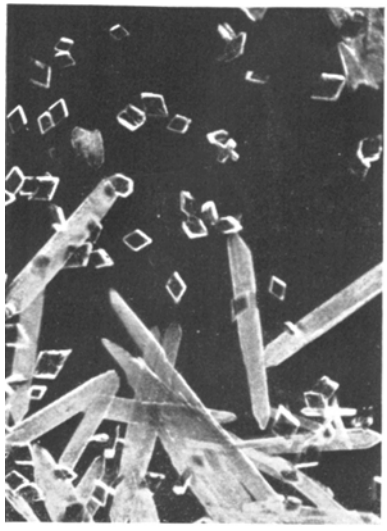


FIG. 1. Amydricaine (Dichromate).

FIG. 2. Amydricaine (Potassium Iodide).

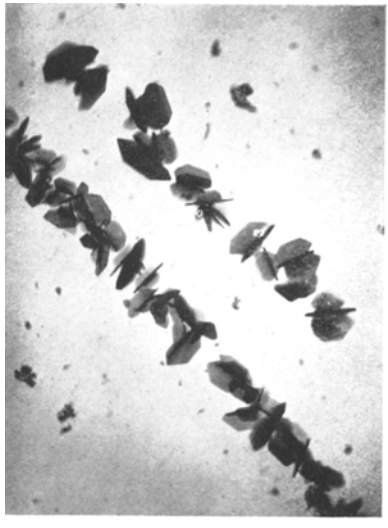


FIG. 3. Amydricaine (Permanganate).

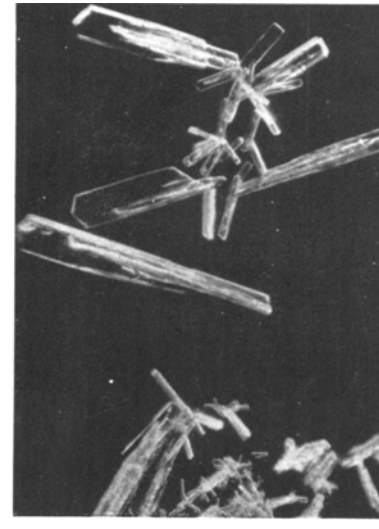


FIG. 4. Amylocaine (Potassium Iodide).

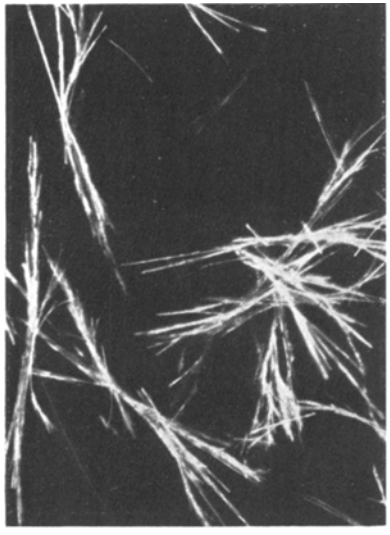


FIG. 5. Amethocaine (Dichromate).

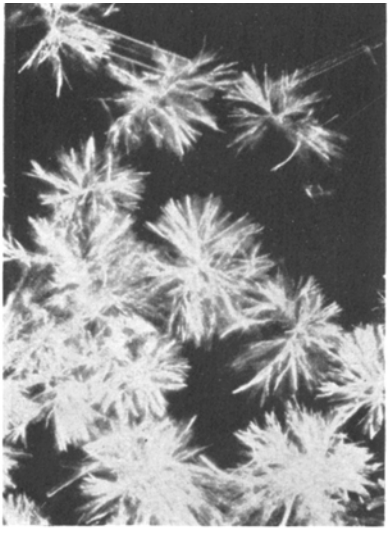


FIG. 6. Amethocaine (Potassium Iodide).

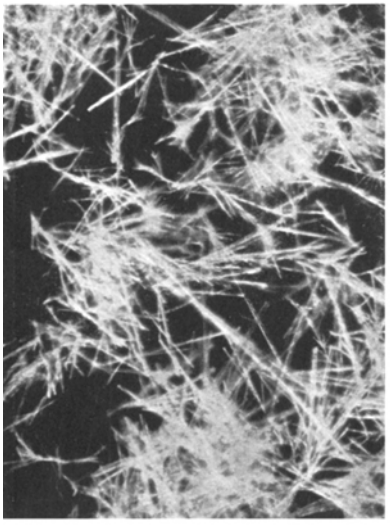


FIG. 7. Butacaine (Potassium Iodide).

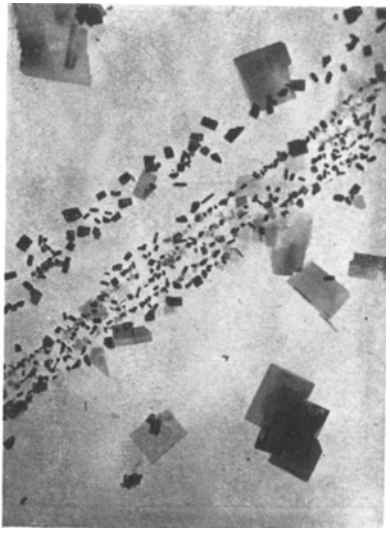


FIG. 8. Cocaine (Permanganate).

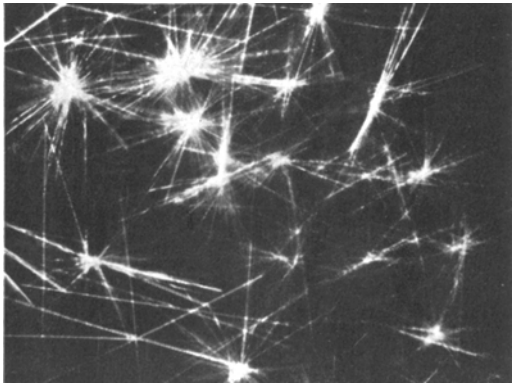


FIG. 9. Benzamine (Potassium Iodide).

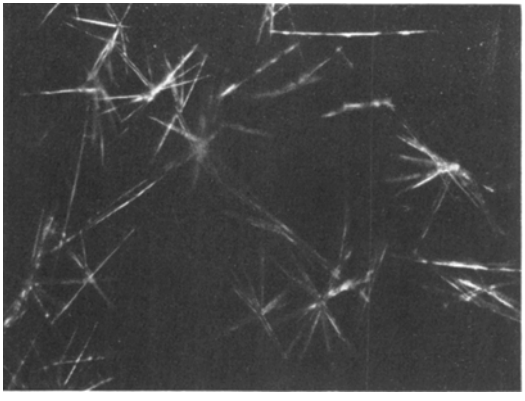


FIG. 10. Metycaine (Potassium Iodide).

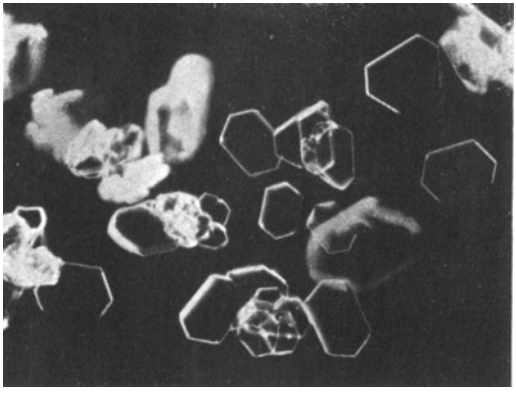


FIG. 11. Procaine (Potassium Iodide).

The magnification of all Figures is approximately × 120

property constitutes a convenient means of purification. Unfortunately, in several instances, notably with procaine, hydrolysis of the ester rapidly occurs during the process, and the initial white precipitate gradually disappears. Care must therefore be taken to avoid addition of any appreciable excess of hydrogen chloride; the following technique has been found to give the most satisfactory results.

The impure basic residue is dissolved in the smallest possible quantity of dry ether, and the solution transferred to a microcentrifuge tube. With the aid of a pipette a few drops of dry ether saturated with hydrogen chloride are carefully added. An immediate white precipitate is obtained which is usually crystalline, but which may initially be of an oily or pasty consistency. After centrifuging, the supernatant liquid is removed, and the residue is dissolved in a minimum amount of alcohol. Addition of ether to this solution causes re-precipitation of the hydrochloride either in crystalline form, or as a gummy product which readily crystallises on standing. From the melting-point of the hydrochloride, taken at this stage, an indication of the identity of the compound is usually obtained (Table I).

In view of the successful results obtained with the local anæsthetic bases, the process is being further investigated as a possible general method for purification of alkaloids and other organic compounds of basic character.

Behaviour with "alkaloidal reagents." Since all the drugs of this group are precipitated by Sonnenschein's phosphomolybdic acid reagent, this test, although in no way specific, is a useful preliminary measure, particularly in the cases of benzocaine and butyl *p*-aminobenzoate, which as indicated above, are extracted chiefly in the "acidic" group. The other common alkaloidal reagents, Mayer's potassio-mercuric iodide, and Wagner's iodine potassium iodide, also provide evidence of a confirmatory nature. In Table II the behaviour of the drugs with these three

TABLE II

REACTIONS OF LOCAL ANÆSTHETICS WITH 'ALKALOIDAL REAGENTS'

	Sonnenschein's Reagent	Mayer's Reagent	Wagner's Reagent
Amethocaine	+	+	+
Butacaine	+	+	+
Procaine	+	+	+
Amydricaine	+	+	±
Metycaine	+	+	+
Amylocaine	±	+	+
Cocaine	+	+	+
Butyl *p*-aminobenzoate	−	−	+
Benzocaine	+	−	±
Orthocaine	+	−	−
Benzamine	+	+	+

reagents is summarised. The tests are most conveniently carried out by dissolving approximately 0·1 mg. of purified hydrochloride in a drop of water, dividing this solution into three separate drops on a black tile, and applying the alkaloidal reagents one to each drop.

Demonstration of benzoic ester structure. The characteristic group

feature of these drugs is the benzoic ester structure. Although no specific test for this typical structure is available, the presence both of an ester and of an aromatic nucleus (with or without amino substituents) may readily be proved (Table III).

TABLE III

REACTIONS FOR THE BENZOIC ESTER STRUCTURE

	Hydroxamic acid reaction (ester linkage)	Alcoholysis (benzoic ester)	Soda lime/furfural reaction (aryl amino group)	Furfural reaction (free—NH_2)
Amethocaine	+	−	+	−
Butacaine	+	−	+	+
Procaine	+	−	+	+
Amydricaine	+	+	−	−
Metycaine	+	+	−	−
Amylocaine	+	+	−	−
Cocaine	+	+	−	−
Butyl *p*-aminobenzoate ...	+	+	+	+
Benzocaine	+	−	+	+
Orthocaine	+	−	+	+
Benzamine	+	+	−	−

(1) 0·1 mg. of the test material is placed in a white porcelain crucible, and to it is added 1 drop of freshly-prepared saturated alcoholic solution of hydroxylamine hydrochloride and 1 drop of freshly-prepared alcoholic potash. The crucible is gently heated until evaporation is complete, when 1 drop of N/2 hydrochloric acid and 1 drop of 1 per cent. aqueous ferric chloride solution are added. A violet-red colour indicates the presence of an ester of a carboxylic acid (Feigl, Anger and Frehden[2]).

(2) 0·1 mg. of material, contained in a test tube (4·5 cm. × 0·5 cm.) is treated with 3 or 4 drops of alcohol and 1 small drop of concentrated sulphuric acid. The tube is warmed over a micro-flame until most of the alcohol has evaporated. Under these conditions alcoholysis results, and the odour of ethyl benzoate is obtained with those compounds in which the aromatic nucleus is unsubstituted.

(3) 0·1 mg. of the test substance, intimately mixed with an equal weight of soda-lime, is heated in a test tube (4·5 cm. × 0·5 cm.) until white fumes are evolved, and an oily distillate approaches to within approximately 0·5 cm. from the open end of the tube. The tube is placed on a white tile, and a pointed strip of filter paper moistened with a 2 per cent. solution of furfural in glacial acetic acid is carefully introduced. A reddish-violet band around the tube in the region of the oily distillate is indicative of aniline bases. Positive reactions are obtained in this test with the amino substituted drugs.

From the results of these three tests an unknown compound may be allocated to this group of drugs with reasonable certainty, and further, within the group, the presence or absence of amino substituents in the aromatic nucleus may be verified. It is recommended that control tests be carried out with typical drugs; under these conditions, and with a little practice, it is possible to utilise even smaller quantities of test material than the 0·1 mg. specified.

One drug only amongst the listed anæsthetics, amethocaine, contains

an aryl amino group which is itself substituted. Whilst, therefore, this compound, in common with the other primary amino derivatives, gives a positive reaction in Test 3 above, it differs from the other members by giving a negative response to the furfural test for a free primary amino group; 0·1 mg. of test material is treated in a white porcelain dish with 1 drop of 2 per cent. furfural in glacial acetic acid, and the liquid allowed to evaporate spontaneously, when a free $-NH_2$ group is indicated by the production of an intense red colour, rapidly turning reddish-violet (Table III).

Crystal and colour reactions. Valuable information as to the identity of a local anæsthetic drug is provided by the following three crystal reactions, which are in many instances completely diagnostic.

(1) *Potassium iodide test.* 0·01 to 0·1 mg. of test material in the form of the hydrochloride is dissolved in a drop of water on a microscope slide; if the material under examination is the free base it is dissolved in a drop of 2N hydrochloric acid. A few particles of finely ground potassium iodide are sprinkled over the surface of the drop, when a positive reaction is indicated by the immediate production of a white turbidity visible to the naked eye. On microscopic examination ($\times 50$) the appearances summarised in Table IV are observed, and for reference purposes the typical crystal forms are given in the photomicrographs.

(2) *Potassium permanganate test.* 0·1 mg. of the drug is dissolved on a microscope slide in a drop of either water or 2N hydrochloric acid as in Test (1). 1 drop of 5 per cent. alum solution is added, followed by a drop of 1 per cent. potassium permanganate solution. After stirring with a glass rod, the slide is examined microscopically ($\times 50$). In Table IV are listed the reactions of the various drugs, whilst Figures 3 and 8 show the characteristic appearances of the only two compounds, amydricaine and cocaine, which yield crystals under these conditions.

(3) *Potassium dichromate test.* 0·1 mg. of material is dissolved on a microscope slide as in the previous tests, and 1 drop of saturated potassium dichromate solution is added. The naked eye and microscopic ($\times 50$) appearances are noted; 1 drop of concentrated hydrochloric acid is then added, and the appearance again observed (Table IV). Although two compounds only, amydricaine and amethocaine, yield crystals (Figs. 1, 5) under the conditions of the test, the colour reactions of the remaining drugs are in many instances quite distinctive.

The drug orthocaine is distinguished from the other benzoic ester local anæsthetics by the presence of a phenolic $-OH$ group. When a crystal of the solid, either base or hydrochloride, is treated with a drop of an aqueous solution of ferric chloride, there is produced a dull blue colour passing to a dull green.

Mixed melting-point determination. The mixed melting-point of the hydrochloride should in all instances be the final test for identity, the

TABLE IV

CRYSTAL AND COLOUR REACTIONS

	Potassium iodide	Potassium permanganate	Potassium dichromate	
			Without concentrated hydrochloric acid	After adding concentrated hydrochloric acid
Amethocaine ...	Dense rosettes of rod-shaped crystals	Amorphous precipitate; violet → greenish-brown	Feathery, yellow needle-growths	Needles disappear giving brownish-green flocculent precipitate
Butacaine	Minute globules giving masses of fine needles	Amorphous precipitate; violet → greenish-brown	Yellow globules	Transient pale violet colour, then brownish-violet globules
Procaine	Minute globules slowly giving large hexagonal plates (crystallisation occurs more readily if a somewhat larger quantity of KI is used)	Amorphous precipitate; violet → greenish-brown	Yellow globules	Violet colour, then brownish-violet flocculent precipitate
Amydricaine ...	Arborescent needle growths	Violet coloured hexagonal plates	Yellow crystals in two forms: rhombs and long flat needles	Crystals dissolve
Metycaine	Minute globules giving large pointed needles tending to rosette formation	Amorphous precipitate; with reddish-brown globules	Yellow globules	Yellow globules unchanged, except for slight darkening
Amylocaine	Thick rods, usually in aggregates	Violet solution slowly depositing brown amorphous precipitate	Yellow globules	Globules dissolve
Cocaine	—	Violet coloured rectangular plates	Yellow globules	Yellow flocculent precipitate
Butyl *p*-aminobenzoate	—	Amorphous brown precipitate	Yellow globules	Violet colour, then brownish-violet flocculent precipitate
Benzocaine	—	Amorphous brown precipitate	—	Brownish-violet flocculent precipitate gradually darkening
Orthocaine	—	Amorphous precipitate; violet → greenish-brown	Bluish-black flocculent precipitate changing to greenish-black	Precipitate gradually dissolves with development of greenish colour
Benzamine	Minute globules giving rosettes of long, very fine needles	—	Yellow globules	Globules gradually flocculate

determination being conveniently carried out in a micro-apparatus of the Kofler or similar pattern.

Summary

1. A scheme is described for the characterisation and identification of the eleven local anæsthetic drugs of the benzoic ester group at present commercially available in this country.

2. The complete scheme is designed in five distinct stages, any of which may be omitted if identification is otherwise satisfactorily achieved:

(i) purification of the crude material by precipitation of the hydrochloride from an ethereal solution of the base;

(ii) reactions with the common alkaloidal reagents;

(iii) demonstration of the presence of the benzoic ester structure;

(iv) simple crystal tests and colour reactions with the three reagents, potassium iodide, potassium permanganate and potassium dichromate.

(v) final identification by micro-mixed melting-point determination.

Acknowledgements

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3. Smith, *Pharm. J.*, 1928, **121**, 88.