THE MICROCHEMICAL DIFFERENTIATION OF MORPHINE AND NALORPHINE

By E. Pedley

North-Western Forensic Science Laboratory, Preston

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Nalorphine (*N*-allylnormorphine) is a drug recently introduced as an antagonist of morphine and other analgesic drugs such as methadone and pethidine. It is therefore liable to be encountered in toxicological and other cases of medico-legal interest in the presence of such analgesic drugs or it may be mistaken for morphine or its compounds under certain circumstances. Thus it is of importance to have a method of distinguishing the two drugs on the micro-scale.

Colour reagents are of little value for this purpose as the colours produced are so similar as to be indistinguishable. This applies to the Marquis, Mecke, Mandelin and Froehde reagents and to the Pellagri reaction and the colours produced with nitric acid. The colour reaction with ammoniacal copper nitrate described by Cooper[1] differentiates the two drugs on the macro scale but the production of the white precipitate is not sufficiently definite in quantities of less than 1 mg. of even pure material to be of practical value with micro quantities.

Absorptiometric methods of differentiating the two drugs have been described. Both show identical maxima and minima in the ultra-violet but they may be distinguished by their infra-red absorption and they may, in fact, be identified in the presence of each other by this method.[2]

An attempt has been made to devise methods of distinguishing between the drugs by microcrystalline reagents, by examination of the parent drugs and derivatives by means of X-ray diffraction and by separation by paper chromatography.

Experimental

Microcrystalline Reagents

A preliminary trial has been made of the reagents shown in Table I using the technique of the Official Methods of Analysis, Association of Official Agricultural Chemists[3], and the following were investigated further: Marme's reagent, Wagner's iodine reagent, picrolonic acid, hydriodic acid and potassium mercuric iodide.

Marme's cadmium iodide reagent. With morphine a silvery-white gelatinous precipitate is produced which rapidly crystallises into masses of fine white needles. Nalorphine produces only an amorphous mass.

Wagner's iodine reagent. With morphine a heavy red-brown precipitate is first formed which slowly forms shining red overlapping crystals extending in plates. Nalorphine produces only a red-brown precipitate and crystals are not formed even after long standing.

Hydriodic acid. As normally encountered this acid (s.g. 1·73) contains much free iodine and if used in this condition the reagent reacts similarly

TABLE I

Reagent	Product of reaction with	
	Morphine	Nalorphine
Marme's	Crystals	Amorphous
Wagner's	Crystals	Amorphous
Picrolonic acid	Crystals	Crystals
Picric acid	Amorphous	Amorphous
Styphnic acid	Amorphous	Amorphous
Kraut's	No precipitate	No precipitate
Mercuric chloride	No precipitate	No precipitate
Hydriodic acid	Crystals	Crystals
Potassium mercuric iodide	Crystals	Amorphous

to Wagner's iodine reagent. If, however, the acid is freed from iodine (by distilling over red phosphorus and using immediately) the crystals obtained are quite different. With morphine long needle-shaped crystals are formed either singly or in bundles or in fan-shaped sheaves according to the concentration (Fig. 1A). With nalorphine short tabular crystals are formed either singly or as agglomerates, again according to the concentration (Fig. 1B). The appearance of the crystals formed with

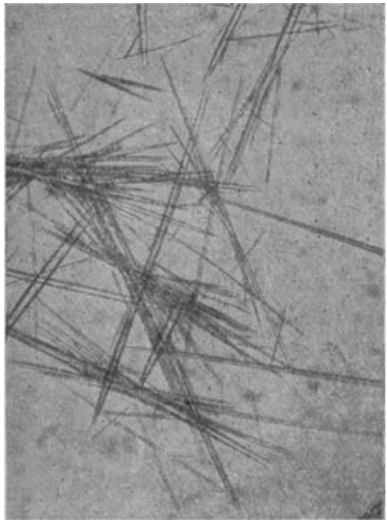

FIG. 1A. Morphine + hydriodic acid.

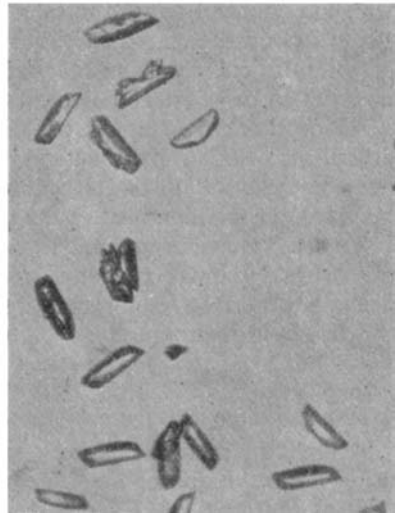

FIG. 1B. Nalorphine + hydriodic acid.

the two drugs is sufficiently striking to permit positive identification and it would seem that this reagent is useful in their differentiation.

Sensitivity of the reaction was determined by placing varying amounts of the drugs on microscope slides (by evaporation of known amounts of their ethanolic solution), dissolving the residues in 0·02 ml. of water or dilute hydrochloric acid and adding 0·03 ml. of iodine-free hydriodic acid. Results are shown in Table II.

The test proved more sensitive for nalorphine than for morphine and is more reliable in that morphine failed occasionally to give crystals

even in concentrations higher than those indicated in the table, whereas nalorphine always gave characteristic crystals with concentrations in excess of 1 in 80.

These experiments also illustrate a weakness of microcrystalline tests, that the form of crystal obtained may vary with the concentration of the drug. In testing tablets and other pharmaceutical preparations it is easy to obtain approximately equivalent amounts of drugs in each test. This cannot apply, however, in dealing with toxicological residues of unknown purity.

TABLE II

Concentration	Morphine	Nalorphine
1:250	No crystals	No crystals
1:125	No crystals	No crystals
1:83·5	No crystals	Occasional crystals
1:62·5	Occasional crystals	Individual crystals
1:50	Sheaves of crystals	Agglomerates
1:25	Large fan-shaped crystals	—

Picrolonic acid. A saturated solution of this reagent in 20 per cent. ethanol was used. With morphine, globular masses were formed which on standing formed bundles of needles (Fig. 2A). More often the globules were more in evidence than the needles. Nalorphine, on the other hand, gives a homogeneous mass which rapidly formed more or less discrete long silky needles (Fig. 2B).

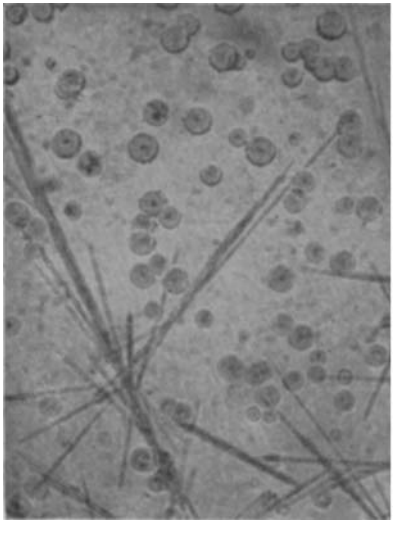

FIG. 2A. Morphine + picrolonic acid.

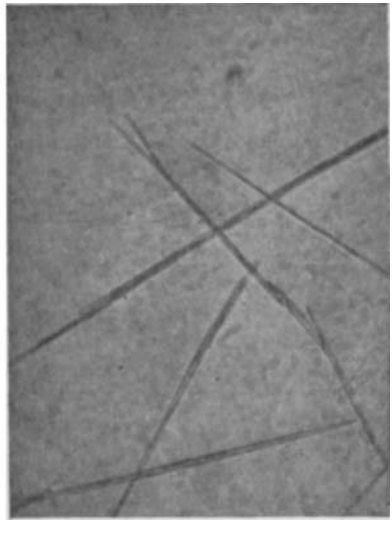

FIG. 2B. Nalorphine + picrolonic acid.

The crystals formed in each case were filtered off, recrystallised and melting point determinations made. The results obtained, however, were erratic and variable and unsuitable for identity purposes. The picrolonates, however, formed excellent crystals for determination by X-ray diffraction (see under).

Potassium mercuric iodide. This reagent has been recommended as a sensitive microcrystalline reagent for morphine[4] using a solution of potassium iodide saturated with mercuric iodide. Experiments confirmed the sensitivity and reliability of the reaction for morphine and the form of the recrystallised morphine–mercuric iodide complex. With nalorphine the reaction is different. The precipitate formed is heavy and amorphous (contrasting with the gelatinous precipitate with morphine) and no crystals were formed even after long standing in a saturated atmosphere for 24 hours. Attempts to crystallise the complex from aqueous acetone yielded a thin yellow oil.

Paper Chromatography

This proved to be a simple and excellent method for separation of the two drugs since they were found to have differing R_F values. Methods of Munier and Macheboeuf[5,6] were applied, using paper treated with 0·5 M potassium chloride and a modified method of Curry and Powell[7] using downward displacement. R_F values obtained are given in Table III. Amounts as low as 25 μg. were detectable and a mixture containing equal amounts (50 μg. of each) separated clearly and gave distinct spots.

TABLE III

	Solvent A R_F	Solvent B R_F	Solvent C R_F
Morphine ..	0·59	0·46	0·22
Nalorphine ..	0·68	0·70	0·39

A: *n*-Butanol–hydrochloric acid 100:2, saturated with water.
B: *n*-Butanol–glacial acetic acid 10:1, saturated with water.
C: *n*-Butanol–citric acid 100:1, saturated with water.

TABLE IV

INDEX LINES

Compound	Strongest lines (*d*) 1st	2nd	3rd	Innermost lines (*d*)
Morphine	6·00	6·60	4·18	10·04
Nalorphine	5·06	7·05	4·40	8·34
Morphine picrolonate	7·13	6·17	3·54	8·89
Nalorphine picrolonate	3·47	5·17	6·13	11·78

TABLE V

X-RAY DIFFRACTION DATA

Morphine (*d*)	Intensity	Nalorphine (*d*)	Intensity	Morphine picrolonate (*d*)	Intensity	Nalorphine picrolonate (*d*)	Intensity
2·10		2·20		3·54		3·00	
2·29		2·58		4·20		3·24	
2·39		3·14		6·17	s.	3·47	vs.
2·71		3·64		7·13	s.	3·71	
3·46	s.	3·84	s.	8·89		3·78	
3·73		4·40	vs.			4·35	
4·18		5·06	vs.			4·58	s.
4·78	s.	5·60	s.			5·17	vs.
5·03	s.	5·92				5·71	
6·00	vs.	7·05	vs.			6·13	
6·60	vs.	8·34	s.			6·68	vs.
7·46	s.					8·15	vs.
10·04						11·78	

s.—Strong. vs.—Very strong.
Data: Phillips Metallix Equipment, Copper radiation (Kα), Nickel filter. Camera diameter, 115 mm.

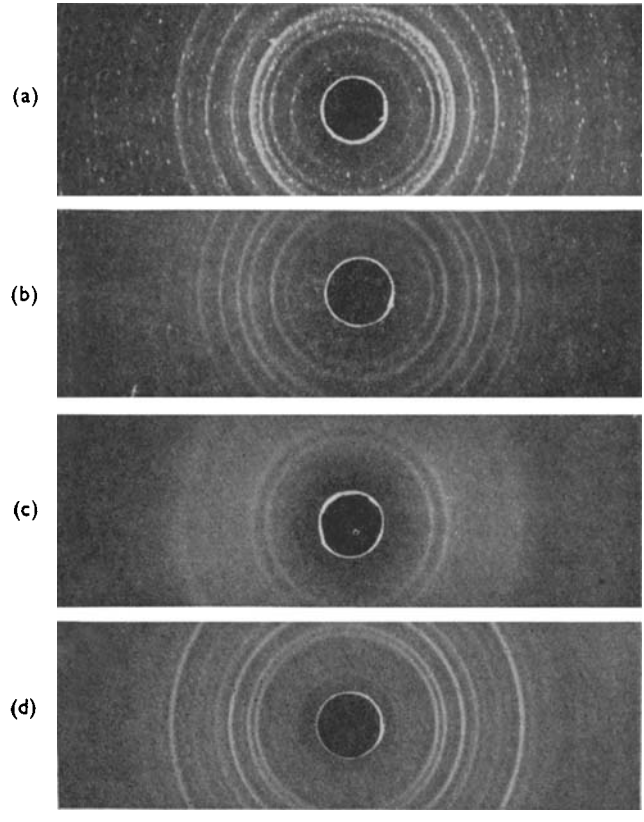

FIG. 3. X-ray diffraction pattern of, (*a*) Morphine, (*b*) Nalorphine, (*c*) Morphine picrolonate, (*d*) Nalorphine picrolonate.

X-Ray Diffraction Patterns

Morphine and nalorphine bases give well-defined X-ray diffraction patterns. These are reproduced in Figure 3. Index lines and diffraction data are given in Tables IV and V.

SUMMARY

1. Micro-methods of differentiating morphine and nalorphine have been investigated.

2. Many microcrystalline reagents give excellent crystals with morphine but failed with nalorphine. Iodine-free concentrated hydriodic acid is a notable exception and is a possible reagent for the identification of morphine. Picrolonic acid is less satisfactory.

3. Excellent separations of the two drugs may be obtained by paper chromatography.

4. X-ray diffraction data of morphine and nalorphine bases and their picrolonates are given.

References

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