PHYSICO-CHEMICAL STUDIES OF (1-METHYL-2-PYRROLIDYL) METHYL BENZILATE METHYL METHOSULPHATE

PART I. THE DETERMINATION OF (1-METHYL-2-PYRROLIDYL)METHYL BENZILATE METHYL METHOSULPHATE IN THE PRESENCE OF ITS BREAKDOWN PRODUCTS

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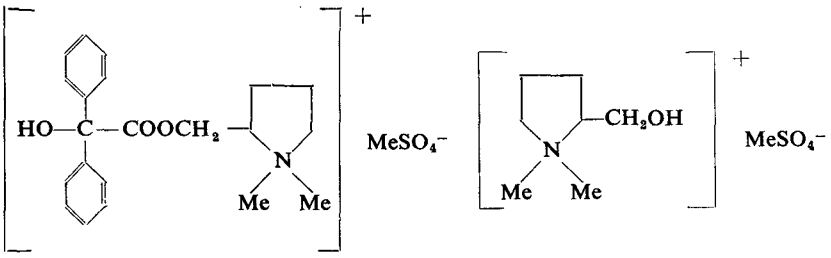
The determination of (1-methyl-2-pyrrolidyl)methyl benzilate methyl methosulphate by the formation of a chloroform-soluble, blue complex with ammonium cobaltothiocyanate is described. The optical density in chloroform at 322 mμ is directly related to the amount of compound present. The method is applicable in the presence of breakdown products and has been used to determine the concentration of (methyl-2-pyrrolidyl)methyl benzilate methyl methosulphate in tablets and in linctuses.

THE ester (1-methyl-2-pyrrolidyl)methyl benzilate methyl methosulphate (I) (poldine methosulphate, Nacton) can hydrolyse to give benzilic acid and *N*-methyl prolinol methyl methosulphate (II). The ultra-violet



I II

absorption spectrum of poldine methosulphate is almost entirely due to the benzilic acid moiety. Attempts to obtain an analytical separation from mixtures with benzilic acid failed. Bromothymol blue[1,2] and Orange II[3] have been used for the extraction and estimation of quaternary ammonium compounds with long side chains. Both the compound and the prolinol moiety react with these reagents.

Brown and Hayes[4] state that cetyltrimethylammonium bromide, but not phenyltrimethylammonium chloride give the reaction with ammonium cobaltothiocyanate described by Gnamm[5], and developed by van der Hoeve[6] and Wurzschmitt[7]. Helgren, Theivast and Campbell[2] used this reaction to determine the quaternary anti-acetylcholine substance "Tral."

It was found that poldine methosulphate but not *N*-methyl prolinol methyl methosulphate reacted with ammonium cobaltothiocyanate.

EXPERIMENTAL

The work of Brown and Hayes[4] is concerned with the determination of polyethylene glycol (PEG) mono-oleates. To 20 ml. of ammonium cobaltothiocyanate reagent is added 5 ml. of a solution of polyethylene glycol mono-oleate. After reaction is complete, the blue complex formed is extracted into chloroform and the optical density read at 318·5 mμ or

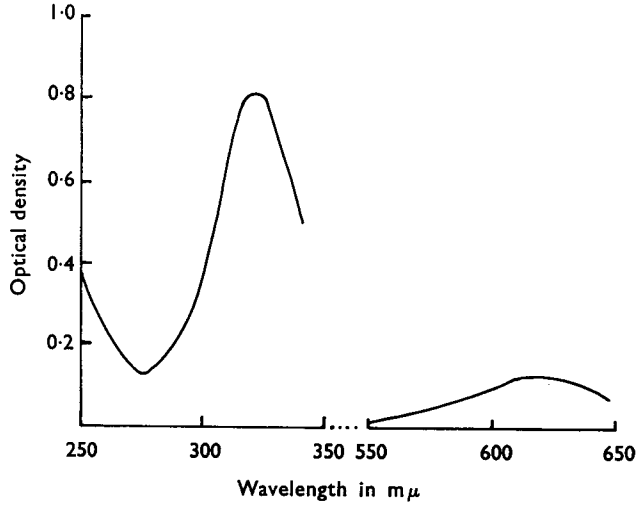


FIG. 1. Absorption curve of poldine methosulphate cobaltothiocyanate complex in chloroform.

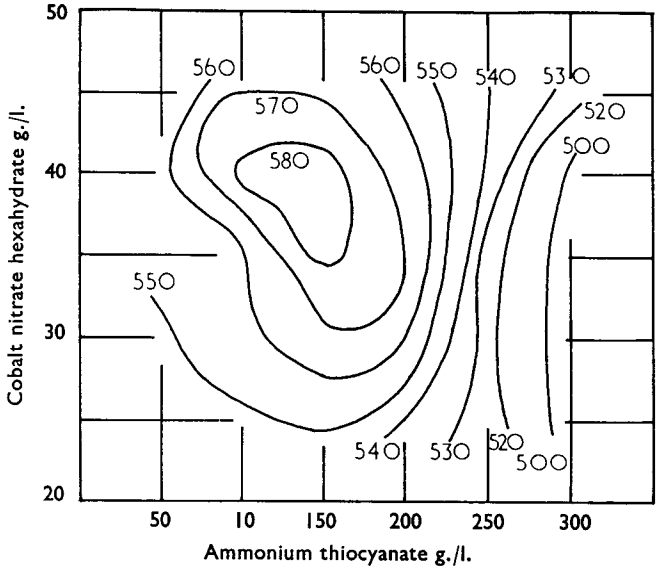


FIG. 2. Contour maps showing optimum cobaltothiocyanate reagent. Contours are optical density × 1,000 for poldine methosulphate.

620 mμ. Under carefully controlled conditions exact determinations could be made by this method.

When we applied this to a specially purified sample of poldine methosulphate prepared by the organic department of the Research Division of Beecham Research Laboratories Ltd., two differences were found. The absorption maxima were at 322 mμ and 620 mμ (Fig. 1). The optimum reagent composition was not the same.

TABLE I

DAY-TO-DAY VARIATIONS IN CALIBRATION CURVES FOR POLDINE METHOSULPHATE

Poldine methosulphate mg.	Optical density at 322 mμ, 1 cm. cell				
	A	B	C	D	E
0·3	0·103	0·105	0·105	0·103	0·104
0·6	0·204	0·227	0·201	0·212	0·225
0·9	0·330	—	0·325	0·332	0·318
1·2	0·451	0·454	0·459	0·459	0·464
1·5	0·574	0·587	0·580	0·579	0·586
Slope of regression line	0·396	0·397	0·403	0·400	0·405
Intercept of regression line	−0·024	−0·014	−0·029	−0·023	−0·026
Value calculated for 1·5 mg. compound 499	0·570	0·581	0·576	0·577	0·582

To determine the optimum reagent composition a series of reagents in which the concentration of cobalt nitrate hexahydrate was varied from 25 to 45 g./l. and ammonium thiocyanate from 50 to 350 g./l. was prepared. To 20 ml. of reagent was added 5·0 ml. of a solution containing 0·3 g./ml. poldine methosulphate. After reaction, the complex was extracted with chloroform and the optical density of the chloroform solution determined at 322 mμ. A contour map (Fig. 2) relating optical

TABLE II

EFFECT OF BREAKDOWN PRODUCTS ON THE ASSAY OF POLDINE METHOSULPHATE

Poldine methosulphate mg.	*N*-methyl prolinol methyl methosulphate mg.	Benzilic acid mg.	Optical density 322 mμ, 1 cm. cell
1·0	—	—	0·381
1·0	1·0	—	0·380
1·0	2·0	—	0·380
1·0	5·0	—	0·380
1·0	—	1·0	0·378
1·0	—	2·0	0·382
1·0	—	5·0	0·382
1·0	5·0	5·0	0·381

density to concentrations of the two components of the reagent was constructed. This compares with that obtained by Brown and Hayes[4]. The contour map shows a peak rather than a plateau, and the differences in optical density are much less. Subsequent work has shown that the type of contour map obtained and the optimum reagent composition varies from compound to compound.

METHOD

Reagents. Ammonium cobaltothiocyanate solution. Dissolve 37·5 g. of Analar cobalt nitrate hexahydrate and 150 g. of Analar ammonium

thiocyanate in water and make up to 1 l. with distilled water. *Chloroform.* Analar, suitable for ultra-violet spectrophotometry.

Procedure. Transfer by pipette 20·0 ml. of ammonium cobaltothiocyanate reagent to a 100 ml. separating funnel. To this, add by pipette, 5·0 ml. of a solution containing 0 to 0·3 mg./ml. of poldine methosulphate. Shake the funnel vigorously for 1 minute and allow to stand for 5 minutes. Add 5 ml. chloroform, shake vigorously for 1 minute and allow to stand for 5 minutes. Swirl the funnel gently to mix the chloroform layer and run off the chloroform layer into a 25 ml. volumetric flask. Extract with a further three portions of 5 ml. of chloroform. Wash the outside of the

TABLE III

DETERMINATION OF POLDINE METHOSULPHATE IN STORED TABLETS

Batch No.	Age months	mg./tablet	
		Declared	Found
E2/134	4	1·0	0·95
	4	1·0	0·98
	4	1·0	0·94
E2/124	2	2·0	1·94
	4	2·0	1·96
E2/150	2	2·0	2·00
	4	2·0	1·96

TABLE IV

DETERMINATION OF POLDINE METHOSULPHATE IN LINCTUSES

Sample	Age	mg./ml.	
		Declared	Found
A	1 year	0·75	0·76
B	1 year	0·25	0·29
C	6 months	0·25	0·27
D	6 months	0·75	0·77
C	1 year	0·25	0·27
D	1 year	0·75	0·77

stem of the separating funnel with chloroform using these washings to make the combined chloroform extracts up to the mark. Shake to mix. Centrifuge the chloroform solution in a stoppered centrifuge tube for 5 minutes at 2,000 r.p.m. to remove water droplets. Measure the optical density of the chloroform solution against a chloroform blank at 322 mμ in 1 cm. cells.

Prepare a calibration curve using 5·0 ml. of solutions containing 0·06, 0·12, 0·18, 0·24, 0·30 mg./ml. of poldine methosulphate. From the calibration curve read off the amount of compound in the original 5 ml. aliquot. An approximate result may be calculated from the equation $y = 0{\cdot}4x - 0{\cdot}025$, where y is the observed optical density and x is the amount of compound in the original aliquot in mg. The equation holds over the range $x = 0{\cdot}3$ to 1·5.

RESULTS

Calibration curve. Table I shows calibration curves obtained on different days with different batches of reagent. The slope and intercept

of the regression line, and the calculated value for 1·5 mg. poldine methosulphate are also shown. The calibration curve is linear over the range 0·3 to 1·5 mg.

Blanks. Helgren, Theivagt and Campbell[2] reported large blanks at the shorter wavelength, using this reagent. Our experience was similar to Brown and Hayes in that negligible blanks were obtained.

Breakdown products. The assay procedure was carried out using 1·0 mg. of the compound with the addition of from 1 to 5 mg. of benzilic acid or *N*-methyl prolinol methyl methosulphate, or both (Table II). No significant difference in optical density between these determinations was found.

Tablets and linctuses. The method has been used to determine poldine methosulphate in stored tablets (Table III) and linctuses (Table IV).

Other substances. Ammonium cobaltothiocyanate did not react with the tertiary amines caffeine, quinine, codeine, ephedrine, atropine, or the unquaternised compound. But reacted with *N*-methylprolinyldiphenylmethyl ether. All these reactions were carried out at the 1·5 mg. level.

Discussion

The ammonium cobaltothiocyanate reagent provides a means of distinguishing between quaternary ammonium compounds of high and low molecular weight and in particular between the ester poldine methosulphate and its parent alcohol *N*-methyl prolinol methyl methosulphate. It has enabled us to demonstrate the stability of the compound in pharmaceutical preparations.

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After Mr. Singleton presented the paper there was a DISCUSSION.