RESEARCH PAPERS

THE QUANTITATIVE SEPARATION OF PAPAVERINE FROM NARCOTINE IN MIXTURES

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Received February 3, 1958

A method for the quantitative separation of papaverine from narcotine in mixtures by using ammonium reinckate is described, and also its application to the determination of these alkaloids in opium. The identity and purity of these alkaloids isolated from opium is shown by means of ultra-violet, visible and infra-red spectrophotometry. The complete extraction of these alkaloids from the triturate of opium by chloroform was demonstrated by paper chromatography. The regeneration of pure papaverine from the reineckate complex is also described.

VARIOUS methods have been proposed for the separation and determination of papaverine and narcotine which are usually isolated from opium in admixture. Plugge[1–3] separated papaverine from narcotine by precipitation of the former with potassium ferricyanide and decomposition of the resulting hydroferricyanide with dilute sodium hydroxide solution. The method gave gummy precipitates when applied to opium[4]. Isolation of papaverine as the acid oxalate was described by Hesse[5] but this procedure is useful only as a means of purification of papaverine.

Annelar reported a method based upon the opening of the lactone ring of narcotine with alcoholic potash to form the soluble potassium narcotinate followed by the extraction of papaverine[6]. A modification of this method was adopted as the official method for the analysis of these alkaloids in papaveretum by the British Pharmaceutical Codex 1954. We found that this method did not give quantitative recoveries even when applied to the determination of mixtures of pure drugs. A brief description of the use of ammonium reineckate for the quantitative separation of papaverine from narcotine has recently been reported by us[8] and it is the purpose of this paper to describe the method in detail.

EXPERIMENTAL

The Separation and Determination of Papaverine and Narcotine in Mixtures of the Drugs

Reagents. (i) Papaverine m.p. 145 to 146°; (ii) Narcotine m.p. 175 to 176°; (iii) Chloroform (Analar); (iv) Acetone (Analar); (v) 0·1N hydrochloric acid; (vi) Ammonium reineckate—approximately 2 per cent solution prepared by dissolving 2 g. of ammonium reineckate in 100 ml. cold water and filtering through a Whatman No. 42 paper. This solution is stable in a refrigerator for about a week and it should be filtered before use if precipitation has occurred; (vii) Crystal violet in 0·5

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per cent solution prepared by dissolving 0·5 g. in 100 ml. glacial acetic acid; (viii) Standard perchloric acid in 0·05N solution is prepared by dissolving about 7·15 g. of 70 per cent perchloric acid in 200 ml. glacial acetic acid. Thirty ml. of acetic anhydride are added and the solution diluted with glacial acetic acid to 1/1. After a day this solution is standardised by the method of the B.P. 1958, page 809; (ix) Silver nitrate—1 per cent aqueous solution.

Procedure. Mixtures of varying amounts of papaverine 6 to 32 mg., and narcotine 30 to 150 mg., are dissolved in 70 ml. of chloroform in 250 ml. flasks fitted with ground glass stoppers. Thirty ml. 0·1N hydrochloric acid and 10 ml. ammonium reineckate solution is then added and the resulting solution is shaken mechanically for 30 minutes. The solution is cooled in the refrigerator for a further 30 minutes and filtered through sintered glass with suction. The papaverine reineckate in the filter is washed with three 5 ml. portions of cold water and the residue dried by suction. The filtrate is set aside for the estimation of narcotine.

Determination of Papaverine

The stem of the funnel containing the papaverine reineckate is rinsed with a little acetone to remove water and is then placed in a second dry suction flask. About 5 ml. of acetone is poured on to the papaverine reineckate. After the reineckate salt has dissolved, the solution is collected under gentle suction. The process is repeated with fresh 1 ml. portions of acetone until the effluent is colourless. The red coloured acetone solution is quantitatively transferred to a volumetric flask and diluted with acetone to exactly 10 ml. or 25 ml. volume, depending on the amount of papaverine reineckate present which is judged by the intensity of the colour of the original solution. The solution is shaken and the optical density determined at 525 mμ in a spectrophotometer using acetone as the blank. The amount of papaverine present can be calculated by means of a calibration curve obtained under similar conditions or by using the following equation:

$$w = \frac{A}{110 \cdot 0} \times M \times \frac{v}{1000}$$

where w = weight of papaverine in mg.
A = observed optical density using one cm. cell.
M = molecular weight of papaverine in g. (339·4).
v = volume in ml. in which reineckate complex is dissolved.
110 = ϵ (gram-molecular extinction coefficient for papaverine reineckate).

Determination of Narcotine

The chloroform : water filtrate obtained after the filtration of papaverine reineckate is transferred to a separating funnel, and the chloroform layer is separated. The suction flask is rinsed with 25 ml. of chloroform which is used to re-extract the alkaloids from the aqueous layer in the separatory funnel. The aqueous solution is then rejected.

The red-coloured chloroform which contains the narcotine is placed in a separatory funnel and to it is added 30 ml. of water and 10 ml. of 1 per cent silver nitrate solution. This solution is shaken until the chloroform layer becomes colourless. The chloroform is separated from the aqueous layer which is further extracted with two fresh 10 ml. portions of chloroform. At this stage vigorous shaking should be avoided to prevent the formation of a suspension of silver reineckate. The combined chloroform fractions are filtered through a funnel plugged with cotton wool. The filtrate is collected in a 250 ml. volumetric flask and made up to volume with chloroform. The narcotine can then be determined by two methods.

Method A—spectrophotometric determination. The optical density at 310 mμ of the chloroform solution or a diluted solution is determined by means of the spectrophotometer. The molecular extinction coefficient of narcotine in chloroform at 310 mμ is 4750.

Method B—determination by non-aqueous titration. An aliquot of the chloroform solution containing at least 20 mg. narcotine is evaporated to dryness in an evaporating dish. The residue is dissolved in 25 ml. of glacial acetic acid and 3 ml. of acetic anhydride and this solution is titrated with standard perchloric acid using crystal violet as the indicator.

Extraction of Papaverine and Narcotine from Opium

Opium 4·5 g. is triturated with 25 ml. of glacial acetic acid for 15 minutes followed by 20 ml. of water, and the resulting mixture is filtered through a Whatman No. 42 paper. This amount of opium is used to provide sufficient volume of filtrate for replicate determinations. A 10 ml. aliquot of the filtrate is extracted successively with 10 ml. portions of chloroform, each of which is passed through a series of separating funnels containing water, sodium hydroxide, sulphuric acid and aqueous sodium bicarbonate solution shown below.

Separatory funnel	Containing
No. 1	Opium filtrate (10 ml. opium solution in acetic acid)
No. 2	15 ml. water
No. 3	15 ml. water and 15 ml. 1 : 1 sodium hydroxide and a few grains sodium bisulphite
No. 4	15 ml. water
No. 5	15 ml. 0·1N sulphuric acid
No. 6	10 ml. 0·1N sulphuric acid
No. 7	10 ml. water and 0·5 g. sodium bicarbonate.

The process is considered completed when two drops of chloroform taken from separatory funnel No. 4 gives no yellow colour when tested for thebaine with syrupy phosphoric acid.

The chloroform extracts are combined (about 70 to 80 ml.) and evaporated on a hot water bath. Unnecessary heating of the dry residue is avoided to prevent decomposition of the alkaloids. The residue is then dissolved in about 50 ml. of carbon tetrachloride and passed through a funnel plugged with cotton wool to remove insoluble impurities. The resulting filtrate is passed through a column of calcium hydroxide using suction which removes further impurities from the carbon tetrachloride

solution leaving the papaverine and narcotine in solution. The calcium hydroxide column is then washed with two 10 ml. portions of carbon tetrachloride. The combined carbon tetrachloride fractions are then shaken with two 20 ml. portions of 0·1N hydrochloric acid in a separatory funnel. A small portion of the carbon tetrachloride solution is then tested for the complete removal of papaverine and narcotine with Frohde's reagent and the persulphate reagent, respectively. When negative tests are obtained this carbon tetrachloride solution is reserved for redistillation.

Determination of Papaverine and Narcotine

The hydrochloric acid fractions from above are combined in a 250 ml. stoppered flask and to it is added 70 ml. of chloroform. This mixture is shaken for 10 minutes and then 10 ml. of ammonium reineckate solution added and the resulting mixture shaken mechanically for 30 minutes. The solution is cooled in the refrigerator for a further 30 minutes and then filtered through sintered glass with suction. The papaverine reineckate which is collected on the filter is dissolved in acetone and determined as described previously.

The narcotine present in the chloroform-water filtrate is also determined in the manner described for the determination of pure drugs.

Discusion of Results

Principles of the Method

The reaction between a base and ammonium reineckate, in acid solution, can be represented by the following equation:

$$BHX + NH_4R \longrightarrow NH_4X + BHR$$

However, narcotine in the presence of excess chloroform does not form an insoluble reineckate, whereas papaverine does, and this difference in chloroform solubility forms the basis of a method of separation of papaverine and narcotine. Table I shows the recoveries obtained using various proportions of papaverine and narcotine.

Under the conditions described earlier, the red coloured chloroform solution obtained after papaverine reineckate had been removed could not be used for direct narcotine determination, since no linear relation was found between concentration and absorbance. Of sixty reineckates which have been studied only narcotine reineckate showed this chloroform solubility behaviour. It was further observed that the red colour attributed to the reineckate can be removed by shaking the chloroform with water. However, the complete removal of the colour could be effected only by shaking the chloroform with silver nitrate solution. The silver reineckate, insoluble in both phases, can be removed by filtration.

The Separation of Papaverine and Naroctine from Opium

The extraction procedure described separates the major opium alkaloids into three groups, (A) morphine and codeine which remain in the acid-containing separatory funnels (1) and (2); (B) thebaine which is retained in the sulphuric-acid-containing funnels (5) and (6); (C) narcotine and

papaverine which are present in the chloroform. They are then determined by the procedure previously described. For ultra-violet absorbance measurements, it was found necessary to remove other ultra-violet absorbing materials present in the solution of the papaverine and narcotine by redissolving them in carbon tetrachloride after evaporation of the

TABLE I

RECOVERIES OF PAPAVERINE AND NARCOTINE FROM MIXTURES

Papaverine and narcotine used		Papaverine (recovered as the reineckate complex)			Narcotine recovered as the free base	
Amount of papaverine used (mg.)	Amount of narcotine used (mg.)	Amount of acetone used in dissolving the reineckate complex (ml.)	Observed optical density "A"	Amount of papaverine recovered (mg.)	Amount of 0·502N aceous perchloric acid used (ml.)	Amount of narcotine recovered (mg.)
6·7	42·4	10	0·218	6·7	2·06	42·5
9·0	65·4	10	0·295	9·1	3·15	65·2
10·8	79·9	10	0·344	10·6	3·86	80·0
11·65	174·4	25	0·152	11·7	8·36	173·2
14·5	32·4	10	0·470	14·5	1·54	31·8
17·0	74·0	10	0·550	17·0	3·52	72·9
20·95	146·4	25	0·269	20·8	7·0	145·0
25·6	101·6	25	0·339	26·2	4·88	101·0
28·6	107·0	25	0·368	28·4	5·18	107·1
32·35	81·1	25	0·423	32·6	3·92	81·1

TABLE II

DETERMINATION OF NARCOTINE AND PAPAVERINE IN OPIUM*

	Narcotine		Papaverine
	Non-aqueous titration	Spectrophotometric determination	
	mg.	mg.	mg.
Indian export sample ..	63·4	62·1	6·2
	63·6	61·7	6·3
Yugoslavian sample ..	63·7	62·8	29·1
	63·9	64·3	29·4

* 10 ml. acetic acid-opium filtrate used equivalent to about 1 g. opium.

chloroform. Further purification was achieved by passing the carbon tetrachloride through a column of calcium hydroxide; using a final back extraction by means of dilute (0·1N) hydrochloric acid to ensure spectral purity of the drugs. At this stage the separation of the papaverine and narcotine was made in the same way as mixtures of the pure drugs. The results shown in Table II illustrate the amounts of papaverine and narcotine recovered from replicate volumes (10 ml.) of the "aliquots" obtained from 4·5 g. of each of two authenticated United Nations opium samples. The agreement between replicates is good. However, the agreement between duplicates has been found to be less satisfactory. This is attributed to irregularities in the initial extraction of opium by acetic acid.

In order to ascertain that complete papaverine and narcotine extraction from the aqueous acetic acid solution has been achieved, paper chromatographic experiments were made on the contents of the various separatory funnels previously listed. The results of this experiment showed the absence of papaverine and narcotine in all the funnels.

TABLE III

RECOVERIES OF NARCOTINE AND PAPAVERINE FROM OPIUM*

Alkaloids	Anneler's method	Proposed method
	mg.	mg.
Narcotine ..	58·3	63·5
Papaverine ..	28·7	29·8

* 10 ml. acetic-acid-opium filtrate used equivalent to about 1 g. opium.

Papaverine and narcotine are not separated by the *iso*butanol-acetic acid-water mobile solvent used for chromatography. They travel close to the solvent front and comprise the leading spot in the mixture. For a comparison of results of papaverine and narcotine recovered by the Anneler method, given in the United Nations document[7], and the proposed method, Table III should be consulted.

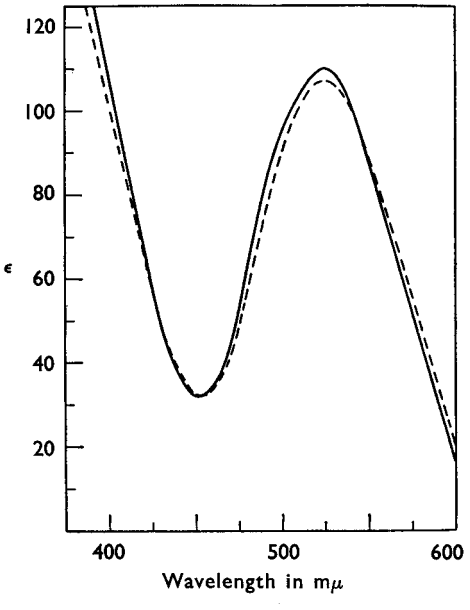

FIG. 1. Ultra-violet curves of papaverine reineckate. — Papaverine reineckate (standard), - - - papaverine reineckate (from opium). Solvent: acetone.

The proposed method gives significantly higher results than Anneler's method which may be explained by loss of narcotine through decomposition. Both alkaloids recovered from the Anneler procedure are found to be spectrally impure. The acetone solution of papaverine reineckate produced by the Anneler method very often has an orange tinge, and the narcotine solution cannot be determined by spectrophotometric means.

Purity of the Extracted Papaverine and Narcotine Fractions

To establish the purity of the papaverine and narcotine separated by this method the physical properties of each drug isolated from opium was established. The spectra of the drugs and their reineckates in the visible, ultra-violet and infra-red regions were measured.

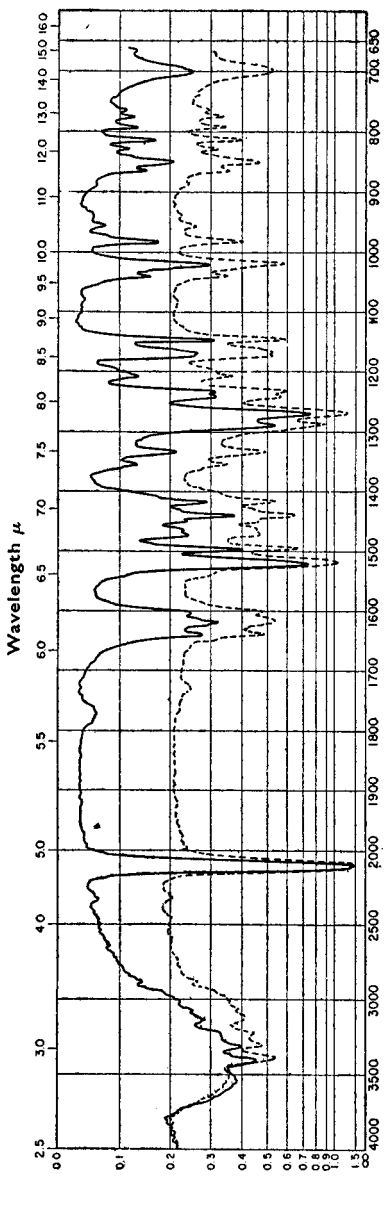

FIG. 2. Infra-red curves for papaverine reineckate. — papaverine reinekate (standard), - - - papaverine reinekate (opium).

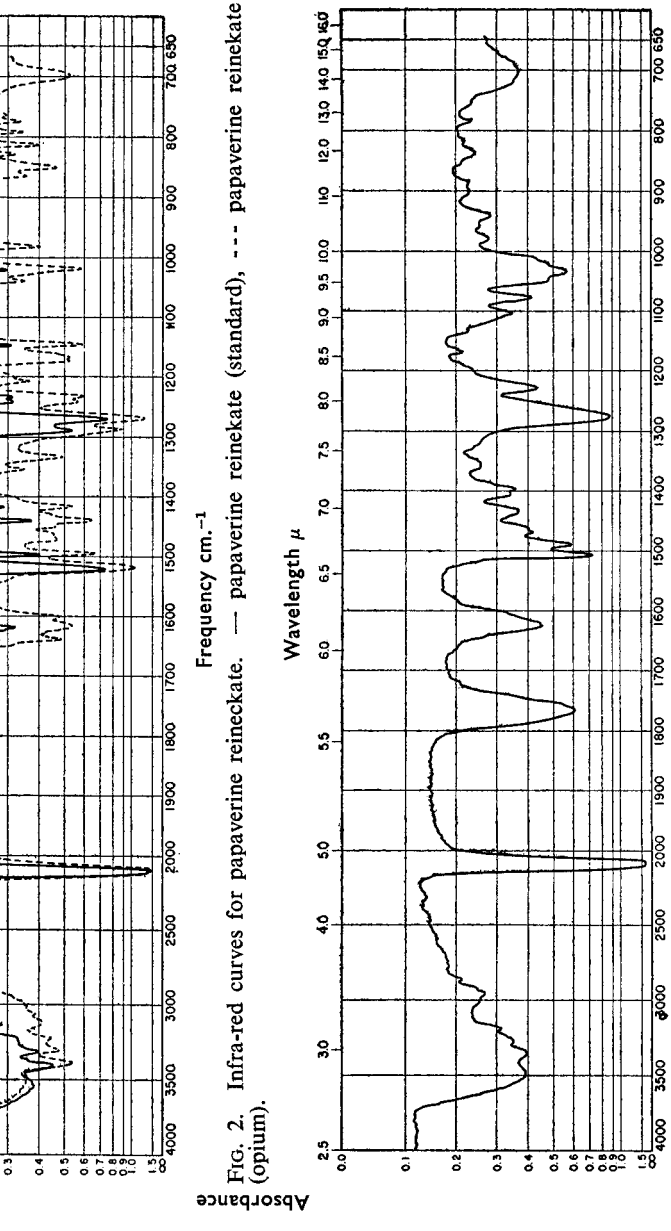

FIG. 3. Infra-red curves of narcotine reineckate.

Spectral Curves of Papaverine Reineckate (Fig. 1)

The ammonium reineckate spectrum (acetone solution) in the wavelength region 350 to 600 mμ is the same when all opium alkaloid reineckates are measured including narcotine reineckate. Indeed, this curve appears to be generally the same for reineckate derivatives of most bases. Papaverine reineckate is exceptional in its spectral behaviour since it shows no maximum in the spectral curve at 395 mμ for papaverine reineckate (Fig. 1).

The infra-red spectra of papaverine reineckate and narcotine reineckate were obtained using the pressed potassium bromide pellets technique. In Figure 2 the infra-red spectra of papaverine reineckates obtained from opium and the pure reference drugs are compared. The two spectra are identical in all features. Figure 3 shows a narcotine reineckate infra-red spectrum obtained from an authenticated sample of the drug. This shows a strong absorption band at 1760 cm.$^{-1}$ which is due to the carbonyl group of the lactone ring. This band is absent in the infra-red spectrum of papaverine reineckate. The possibility of using infra-red spectrophotometry for the quantitative estimation of mixtures of papaverine and narcotine is also shown.

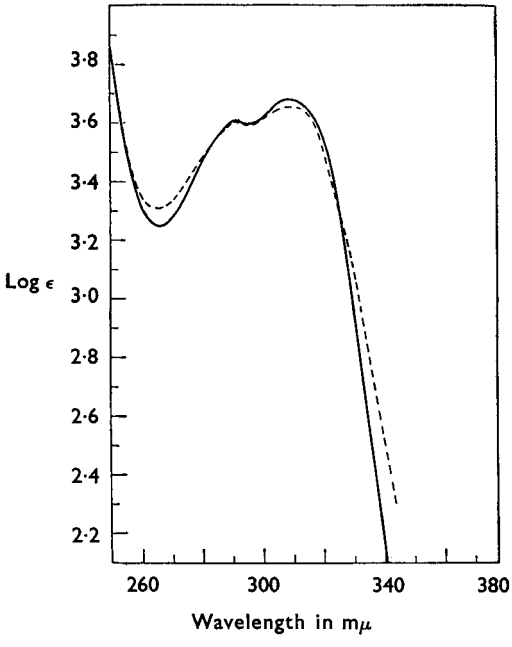

FIG. 4. Ultra-violet curves for narcotine. — Narcotine (standard), - - - narcotine (from opium). Solvent: chloroform.

The Ultra-violet Spectrum of Narcotine from Opium

In Figure 4 the purity of narcotine in the chloroform solution obtained after the separation of papaverine from narcotine by this method is shown. The spectral curves were obtained by means of a Cary (Model 11 N) recording spectrophotometer. To obtain the concentration of narcotine in the chloroform, a non-aqueous titration of a portion of the chloroform for narcotine was made. This concentration was used for calculation of the logarithm of the molecular extinction coefficient (log ϵ) employed in Figure 4. The standard narcotine log wavelength curve in chloroform is also shown. The spectra are practically identical. The absence of additional maxima or minima in the spectral curve of the opium isolate compared with the standard is a clear indication of its purity.

Isolation of Pure Papaverine and Narcotine from Opium

Pure narcotine and papaverine were extracted from opium by the method described. To obtain pure narcotine the chloroform solution containing the alkaloid after the separation of the papaverine was evaporated and the resulting crystals were dissolved in ethanol (95 per cent) and recrystallised twice. The melting point (175 to 176°) was determined by means of the Fisher John's melting point apparatus. A mixed melting point with standard narcotine showed no depression.

Pure papaverine was obtained from the reineckate by treating an acetone solution of the latter with silver nitrate and filtering the resulting mixture. The aqueous-acetone filtrate containing papaverine was extracted with chloroform. Evaporation of the chloroform gave a residue which on recrystallisation with ethanol yielded pure papaverine base m.p. 145 to 146° alone and mixed with an authenticated sample. The papaverine thus obtained gave no colour with concentrated sulphuric acid which indicates the absence of cryptopine.

Acknowledgements. The Senior author acknowledges the financial assistance of the United Nations Technical Assistance Administration and the Singapore government. We wish to thank Drs. Morrell and Pugsley for their interest in this work which was undertaken as part of the U.N. Opium Research Training plan. The assistance of Mr. G. Morris and the biological photographic laboratory in preparing the illustrations is acknowledged.

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