THE DETERMINATION OF THE ORIGIN OF OPIUM

PART II. SIMULTANEOUS ASSAY OF NARCOTINE, THEBAINE AND PAPAVERINE IN OPIUM BY INFRA-RED SPECTROSCOPY*

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A rapid and reliable method for the simultaneous determination of the percentage of narcotine, thebaine and papaverine in opium by an infra-red method has been developed. Examination of the infra-red spectrum of the carbon tetrachloride solution of the dried chloroform residue from an acetic acid-water opium solution revealed a quantitative relationship between the absorbances at 1767, 1602 and 1160 cm.$^{-1}$ and the concentration of narcotine, thebaine and papaverine.

A RECENT international conference of a committee of experts on opium origin determinations at the United Nations[1] recommended that research on opium be continued in order to obtain rapid, reliable and reproducible quantitative analytical values for the major alkaloids contained in opium. They pointed out that the main disadvantage of present unified methods of analysis of opium[2–7,12] is the long time required in the separation and purification of each alkaloid before assay. The simultaneous quantitative determination of papaverine and oxycodone (which contains a C = O group) by infra-red spectroscopy of chloroform solutions was made in 1955[8]. The possibility of simultaneous determination of narcotine, thebaine and papaverine became evident in this laboratory, when it was found that their infra-red spectra in chloroform[9], and carbon tetrachloride showed quantitative relationships between concentration and absorbances at 1767, 1602 and 1160 cm.$^{-1}$ respectively. Further evidence of the possibility of using infra-red analysis for the quantitative estimation of mixtures of papaverine and narcotine was shown and later the determination of narcotine in the presence of papaverine in mixtures of pure drugs was reported[10,11]. It is the object of this paper to describe an infra-red method for the simultaneous assay of narcotine, thebaine and papaverine in opium.

EXPERIMENTAL

Reagents

Reference Standards: Narcotine (100 mg.), (B.P.C. grade) thebaine (70 mg.) and papaverine (50 mg.) (B.P.C. grade) were dissolved in anhydrous carbon tetrachloride and made up to 25 ml. in individual volumetric flasks and stored in the dark.

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Phosphoric acid: 85 per cent.

Persulphate test solution: potassium persulphate (0·1–0·12 g.; reagent grade) was dissolved in concentrated sulphuric acid (5 ml., 95·5 per cent).

Froehde's (fuming) test solution: Dissolve sodium molybdate (0·5 g.) in sulphuric acid (5 ml., 95·5 per cent and dilute with fuming sulphuric acid in the proportion of 1 to 15.

Preparation of Opium Extract[12]

Opium (4·5 g.) powdered and sieved to 20:40 mesh size particles was triturated with glacial acetic acid (20 ml.) to a smooth paste (10–20 minutes rubbing) in a mortar, to which was added water (25 ml.) from a burette, slowly and with continuous stirring. The aqueous acetic acid-opium mixture was filtered through a Whatman 42H filter paper into a narrow graduated cylinder to prevent excessive evaporation. An aliquot (10 ml.) of the opium solution equivalent to 1 g. of opium was taken for analysis and extracted with chloroform according to the scheme given in Table I.

Group separation of Narcotine, Thebaine and Papaverine for Infra-red Analysis

Five clean, dry separatory funnels (125 ml.) were taken and their stop-cocks greased with Apiezon M, or Lubriseal, then washed with chloroform to remove excess grease and assembled on a rotating table. An aliquot (10 ml.) of opium extract was placed in funnel (1). Water, sodium hydroxide, etc. were added to funnels (2) to (5) as shown in Table I.

TABLE I

ARRANGEMENT OF SEPARATORY FUNNELS FOR EXTRACTION OF NARCOTINE, THEBAINE AND PAPAVERINE

Number of funnel	Contents of funnel	Quantity used
(1)	Acetic acid-water-opium solution ..	10 ml.
(2)	Water	10 ml.
(3)	Sodium hydroxide water (1:1) ..	15 ml.
	Water	10 ml.
	Sodium bisulphite	50 mg.
(4)	Water	10 ml.
(5)	Water	10 ml.

The contents of funnel (1) were shaken with chloroform (10 ml.), the layers separated, the chloroform transferred to funnel (2) and the shake-out repeated. At the same time another portion of fresh chloroform (10 ml.) was added to funnel (1). The shake-outs were continued until 4 portions of chloroform had been successively transferred into funnel (1) to (5) in turn. At funnel (2) a test for thebaine was made, using two drops of the chloroform layer, with syrupy phosphoric acid with which it gives a golden yellow colour. Further chloroform extractions were made until the thebaine test was negative. Tests for narcotine and papaverine using persulphate in concentrated sulphuric acid (red orange colour) and fuming Froehde's reagents (intense violet colour) were then made.

The chloroform solution from funnel (5) was filtered through a cotton wool pad, and after evaporation of the chloroform, the residue was dried in a desiccator and triturated with anhydrous carbon tetrachloride. The resulting solution was filtered and washed through a sintered glass funnel under reduced pressure and made up to volume in a volumetric flask (25 ml.) with anhydrous CCl_4. The infra-red spectrum of a portion of this solution was obtained using a 1 mm. NaCl cell by the usual techniques in a Perkin Elmer Model 21 recording spectrophotometer. Chemical determinations of opium alkaloids were made by the modified K/34 method[11,12].

Results and Discussion

Group Separation of Narcotine, Thebaine and Papaverine and Minor Phenolic Alkaloids for the Infra-red Analysis

The filtered solution of the acetic acid extract of opium alkaloids plus other extractives (i.e., coloured materials, etc.) was shaken with chloroform, which divides the alkaloids into two main groups, those readily extracted by chloroform from unbuffered acetic acid solution, and those not readily extractable. The former group consists of narcotine, papaverine, thebaine and about one third of the total amount of minor phenolic alkaloids including nearly all of the porphyroxine-meconidine. The other group remaining in the aqueous solution consists of morphine, codeine, cryptopine, an unknown base, narceine, and about two thirds of the minor phenolic alkaloids.

The chloroform extracts are washed with unbuffered acetic acid solution and then with alkali solution. The chief purpose of which is to remove the acetate ions. The minor phenolic alkaloids that are readily extracted from unbuffered acetic acid are not held by alkali against chloroform.

The complete separation of the group of alkaloids, thebaine, papaverine and narcotine and minor phenolic substances, is indicated by the completion of the thebaine extraction, since narcotine and papaverine are removed from the aqueous phases more readily. The unknown base, which may be extracted slightly, yields a thebaine-like colour rection with sulphuric acid (2 + 1), a slight reaction with syrupy phosphoric acid, and scarcely any colour with concentrated hydrochloric acid. However the latter reagent does not react well with minute amounts of thebaine. A little experience indicates the completion of the thebaine extraction using syrupy phosphoric acid as the main test solution.

The minor phenolic alkaloids are insoluble in carbon tetrachloride and remain in the filter bed after trituration of the chloroform residue of the groups of alkaloids.

Calculation of Concentrations of Narcotine, Thebaine and Papaverine from Infra-red Spectra

The infra-red spectra of pure narcotine, thebaine and papaverine individually and in a mixture are shown in Figure 1. The absorbance

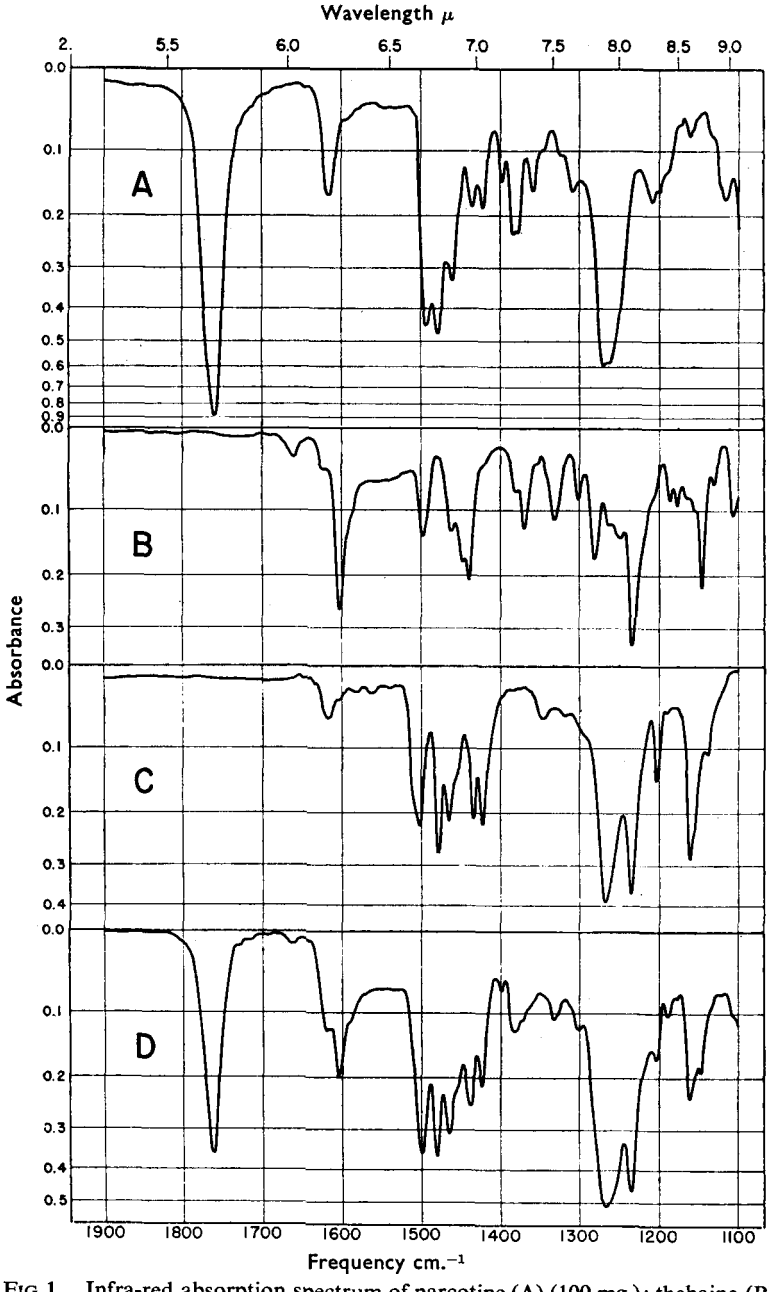

FIG 1. Infra-red absorption spectrum of narcotine (A) (100 mg.); thebaine (B) (70 mg.), papaverine (C) (50 mg.); mixture (D) of narcotine (38·2 mg.); thebaine (38·2 mg.); papaverine (29·3 m.g.) in CCl_4 (25 ml.)

TABLE II

ABSORBANCES AT CHARACTERISTIC FREQUENCIES OF STANDARD PURE BASES

Alkaloid	Concentration mg./25 ml. CCl_4	Absorbances at frequencies, $cm.^{-1}$								
		A_{1767}	A_{1850}	$A_{1767}-A_{1850}$	A_{1602}	A_{1645}	$A_{1602}-A_{1645}$	A_{1160}	A_{1172}	$A_{1160}-A_{1172}$
Narcotine	100	0·900	0·020	0·880	0·065	0·000	0·065	0·078	0·058	0·020
Thebaine ..	70	0·010	0·000	0·010	0·265	0·012	0·253	0·083	0·070	0·013
Papaverine	50	0·015	0·000	0·015	0·040	0·000	0·040	0·288	0·048	0·240

data of Table II were derived from the spectra of these pure alkaloids. From the absorbance data, the following equations were derived for the calculation of percentages of narcotine, thebaine and papaverine:

Per cent narcotine =

$$\frac{(A_{1767} - A_{1850}) - \frac{0{\cdot}01}{0{\cdot}0253}(A_{1602} - A_{1645}) - \frac{0{\cdot}015}{0{\cdot}240}(A_{1160} - A_{1172})}{0{\cdot}00880} \times \frac{100}{1000}$$

Per cent thebaine =

$$\frac{(A_{1602} - A_{1645}) - \frac{0{\cdot}065}{0{\cdot}880}(A_{1767} - A_{1850}) - \frac{0{\cdot}040}{0{\cdot}240}(A_{1160} - A_{1172})}{0{\cdot}00361} \times \frac{100}{1000}$$

Per cent papaverine =

$$\frac{(A_{1160} - A_{1172}) - \frac{0{\cdot}020}{0{\cdot}880}(A_{1767} - A_{1850}) - \frac{0{\cdot}013}{0{\cdot}253}(A_{1602} - A_{1645})}{0{\cdot}00480} \times \frac{100}{1000}$$

The first term of each equation is the net absorbance of the "peak" for each alkaloid measured above a base line. The next two terms are minor corrections for the other two alkaloids. The denominator is based on the net absorbances per milligram obtained from the standard solutions of pure alkaloids, while the final factor converts the calculated concentration from milligrams to per cent.

Figures 2 and 3 show the characteristic features of the infra-red spectra of carbon tetrachloride solutions of chloroform residues from opium samples of two different countries of origin. Figure 2 is the spectrum obtained from an Indian export opium having very low papaverine, and average thebaine and narcotine content, while Figure 3 shows the spectrum of an Iranian type opium with high papaverine and thebaine, and average narcotine content. Table III lists the infra-red data obtained from Figure 2 along with the absorbance correction and sample calculations of alkaloid content.

Comparison of the K/34 and Infra-red Methods

Quantitative results. Table IV lists results of assay by the infra-red method and the comparative assay. The sample of Indian opium

Ex 3398, which has been adopted as a comparison sample in all this work was analysed by the K/34 unified chemical method of analysis described by Fulton in the United Nations document[12]. Samples F112, F113,

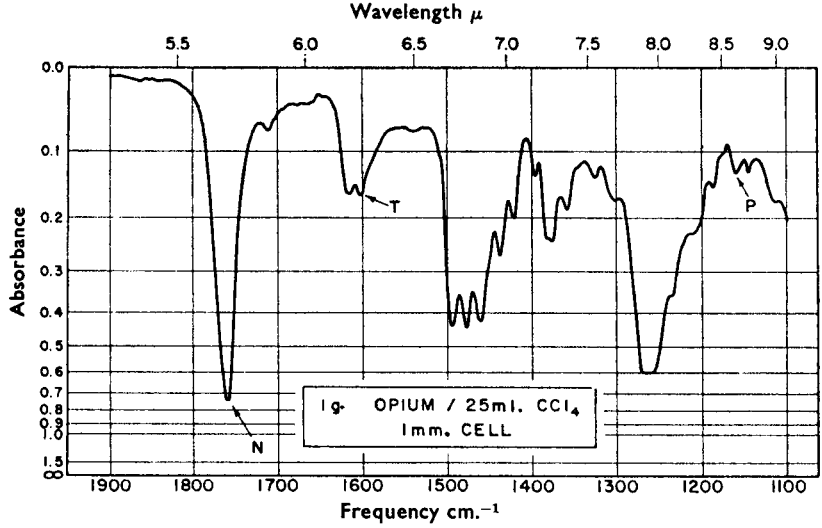

FIG. 2. Infra-red absorption spectrum of extract of Indian export opium reference standard.

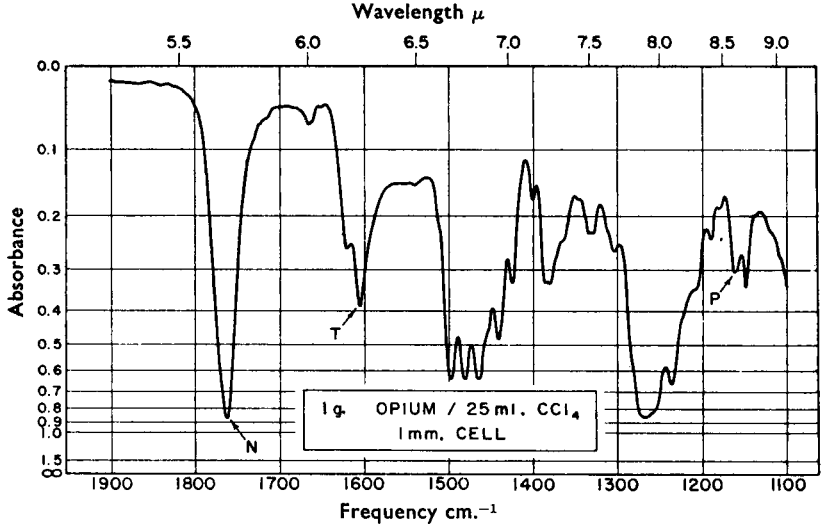

FIG. 3. Infra-red absorption spectrum of extract of Iranian opium (F112).

F119 and F120 which were supplied to us for origin determinations were assayed by a modification of the K/34 method[11]. In addition, a study of the variance of the K/34 method using the Indian export sample Ex 3398, on the results from 8 determinations by 4 different analysts on 4 replicate

samples analysed in duplicate by each analyst was made. The analysts worked at the same time under the same conditions to obtain these data: The modification of the K/34 method[11] was then made and by comparison, yields similar results to the K/34 method[12]. Seven samples were analysed

TABLE III

EXAMPLE OF CALCULATIONS OF ALKALOID PERCENTAGES FROM FIGURE 2

Alkaloid	Peak		Base line		Correction for other alkaloids	Net absorbance	Factor	Per cent
	Frequency $cm.^{-1}$	Absorbance	Frequency $cm.^{-1}$	Absorbance				
Narcotine ..	1767	0·726	1850	0·015	−(0·005+0·003)	0·703	$\frac{100}{0·0088 \times 1000}$	7·99
Thebaine ..	1602	0·167	1645	0·031	−(0·053+0·006)	0·077	$\frac{100}{0·00361 \times 1000}$	2·13
Papaverine ..	1160	0·129	1172	0·090	−(0·016+0·006)	0·017	$\frac{100}{0·00480 \times 1000}$	0·35

TABLE IV

COMPARATIVE RESULTS OF ANALYSIS OF OPIUM SAMPLES BY INFRA-RED AND CHEMICAL METHODS

Opium Sample No.	Content of alkaloid, per cent					
	Narcotine		Thebaine		Papaverine	
	IR[a]	Chem[b]	IR	Chem	IR	Chem
F112	8·50	8·83	6·15	6·26	2·02	2·31
(UN 197)	8·58		6·70		2·39	
F113	8·76	7·02	5·79	6·05	2·29	2·19
(UN 198)	9·04		5·76		2·35	
F119	9·74	8·69	1·21	1·07	4·25	4·01
(UN 226)	9·51		0·95		4·25	
F120	5·42	5·11	2·30	2·43	1·33	1·43
(UN 227)	5·55		2·71		1·30	
Indian ..	7·99	7·8	2·13	2·10	0·35	0·42
Ex 3398	7·58		1·97		0·25	

a IR = Infra-red method of assay.
b Chem = Chemical methods of assay cited in references 11 and 12.

TABLE V

COMPARISON OF 95 PER CENT CONFIDENCE LIMITS FOR NARCOTINE, THEBAINE, PAPAVERINE

Method	Confidence limit for		
	Narcotine	Thebaine	Papaverine
K/34 ..	± 1·27 (10)	± 0·56 (17)	± 0·37 (9)
IR	± 0·40 (7)	± 0·19 (7)	± 0·18 (7)

• Numbers in brackets are degrees of freedom.

in duplicate by the infra-red method beginning with the same Indian sample. All data were submitted to the Biometrician[13] for statistical analysis to establish the confidence limits shown in Table V.

Steps of the K/34 method. For purposes of discussion of the advantages of the infra-red method compared with the K/34 method and its modification, a brief description of the general steps is given.

1. Initial extraction of the raw opium to prepare a solution containing all of the opium alkaloids.

2. Separation of the alkaloids into three groups, (A) morphine and codeine, which remain in acid containing funnels; (B) thebaine which is retained in sulphuric acid containing separatory funnels; (C) narcotine and papaverine which are contained in the chloroform.

3. Countercurrent extraction and separation and purification of morphine, codeine, thebaine, papaverine, narcotine, narceine and an unknown base along with minor phenolic alkaloids.

4. Assay of the purified alkaloids by aqueous or non-aqueous titration.

The K/34 method requires fifty-six separatory funnel solutions and an average of six to eight solvent passes per funnel to remove the alkaloids. The modifications introduced reduce the number of separatory funnels to 20 and thereby the time of analysis appreciably.

Time required for analysis. The time required for the determination of morphine, codeine, thebaine, papaverine and narcotine by the K/34 method is five days per sample. For the modified K/34 method 3 days per sample is required. Determination of only thebaine, narcotine and papaverine by the modified K/34 method takes two days per sample compared with half a day per sample by the infra-red method. Since a large number of samples of opiums will be required to be analysed to meet the needs of the UN opium research programme the time saved is an important consideration.

Reproducibility of the K/34 and infra-red methods. A study of the causes of variance of the K/34 method has been made by Farmilo and others (in preparation). The chromatographic purity of isolated alkaloids, and the amounts in replicate samples in a specially prepared and accurately sampled Indian export opium (T. & H. Smith Ex 3398) were found. The 95 per cent confidence limits for each alkaloid value were determined by statistical analysis of the data[13]. In the infra-red study, confidence limits for the mean values were obtained using the same Indian export opium sample. These two sets of data are compared in Table V and the number of degrees of freedom are shown in brackets after the limit values. It can be readily seen that the infra-red method shows a distinct improvement in reproducibility of results.

Study of Substances with Possible Interfering Infra-red Absorbances

Stopcock grease. It was observed in preliminary experiments after the chemical extraction, when Celloseal* grease had been used on the stopcocks of the separatory funnels shown in Table I, that the narcotine lactone carbonyl band at 1765 cm.$^{-1}$ sometimes showed a shoulder at 1740 cm.$^{-1}$. This indicated additional C = O (not lactone) absorption. A spectrum of the Celloseal grease in carbon tetrachloride showed strong absorption at 1737 cm.$^{-1}$. The spectra of Apiezon-M† and Lubriseal‡

* Stopcock grease sold by Fisher Scientific Supply Co. Montreal, P.Q.
† Edwards High Vacuum, (Canada Ltd) P.O. Box 515, Burlington, Ontario.
‡ Arthur H. Thomas, Supply House, Philadelphia, U.S.A.

greases were free from carbonyl absorption and were satisfactory for this purpose.

Opium fat. The infra-red spectrum of the naturally occurring light petroleum solubles in opium was obtained in carbon tetrachloride solution. This spectrum resembled the spectra of glycerides in general and had a strong absorbance band at 1725 $cm.^{-1}$ which was absent in the spectra of the alkaloid extracts obtained in the usual way. Presumably the glyceride material is removed during the filtration from the initial aqueous acetic acid opium extract. The Indian export opium sample, before and after fat extraction with light petrol yielded identical infra-red spectra.

Secondary alkaloidal constituents. Minor phenolic alkaloid fractions obtained from the study of chemical methods[11] (also paper in preparation) were used to demonstrate the lack of interference of these secondary alkaloids with the narcotine, thebaine, and papaverine during infra-red analysis. A carbon tetrachloride extract of the combined minor phenolic alkaloid fractions was made. An infra-red spectrum of this solution showed little absorbance over the entire wavelength range.

Colouring matter in opium. The carbon tetrachloride solutions of the chloroform residues from the opium extractions are yellow to yellow brown in colour. This colour may be removed by passing the carbon tetrachloride solution through a bed of calcium hydroxide,with some loss of narcotine. An infra-red spectrum of the decolourised solution showed a lower narcotine absorbance, but otherwise was identical with a spectrum of the coloured solution. The colouring matter is not present in the carbon tetrachloride solution in concentrations which affect the infra-red spectrum.

Other major alkaloid constituents. It has been found that codeine is very slightly extracted by chloroform from aqueous acetic acid opium solutions during chemical separation of the thebaine, papaverine and narcotine from morphine and codeine. A study of the chromatograms of concentrated carbon tetrachloride solutions of chloroform residues showed negligible quantities of codeine. This quantity does not affect the characteristic infra-red absorbances of narcotine, thebaine and papaverine. No other alkaloids could be detected chromatographically in these residues.

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