SEPARATION AND IDENTIFICATION OF MAJOR ALKALOIDS FROM OPIUM BY PARTITION CHROMATOGRAPHY ON PAPER*

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Several investigators[1,2,4-8,12-19,21] have used paper chromatography as a means of separating and identifying alkaloids derived from opium. Most methods which have been published, however, utilized pure solutions of alkaloids and are not necessarily applicable to the crude extracts of raw opium, although ASAHINA AND ONO[1] have determined morphine and codeine individually in the natural product.

Both the descending and ascending methods of paper partition chromatography have been studied, but, according to GORE[5], the latter technique may be preferable since it is easier and requires no special apparatus. It has been found that, in general, alkaloids are more sharply resolved on acid buffered paper[16,19]. MUNIER[16] claims that this treatment allows the use of neutral solvents in many instances, and may eliminate most of the trailing and elongation of spots. Increasing acidity will also increase the range of distribution of alkaloids, the optimum being between pH 4.0 and 5.0 for opium alkaloids and their derivatives[12,19].

It appears that the choice developing solvents for alkaloids are the higher alcohols of the aliphatic series (*n*-butanol, isobutanol, amyl alcohols), although other solvents have been used advantageously. For instance, LUSSMAN *et al.*[11] employed cyclohexanol for the separation of cinchona alkaloids. The higher alcohols are usually saturated by shaking or refluxing with water or aqueous solutions of acids (mineral or organic) until equilibrium is reached between both phases. This may require many hours. The water-immiscible solvent is then used for chromatographic development.

Neutral solvents usually show poor resolution[5] unless acid-buffered papers are used[16]. Alkaloids of the opium group and their synthetic derivatives tend to separate more sharply in acidified solvents[18,25], although an excessive concentration of acid in the solvent phase may lead to poor resolution. Under these conditions, the alkaloids have a tendency to follow the solvent front[16]. MUNIER[17] has separated many alkaloids by varying the concentration of acid in the solvent phase, in presence of a minimum of water. Water is added dropwise with constant agitation to a given mixture of organic solvent (say, *n*-butanol, 100 parts) and of acid (acetic acid, 20 parts) until a slight turbidity persists for a while. The solvent may be known as *n*-butanol–acetic acid 5:1. According to the author, the amount of water added need not be indicated since it is exactly that required to saturate the organic solvent and may be duplicated accurately in subsequent preparations. Other advantages of this procedure are that the solvent may be used at once and that the concentration of acid in the solvent phase may be exactly ascertained.

Water-miscible systems (ethanol, propanol, acetone) have also been investigated, but with little success for opium alkaloids[5,6,18]. RESPLANDY[20] claims that some alkaloids can be separated by development with electrolyte solutions. He found that salts of monovalent cations have a retarding action on the motion of the spots, ammonium ion showing the most marked effect. Among the anions, sulfate appears to be most suitable for this purpose.

Most of the methods using water-immiscible solvents also involve development in an atmosphere saturated either with the solvent phase, an aqueous phase, or both, to insure equilibrium of the system. Various types of air-tight containers have been described for this purpose and some are commercially available. In such systems, it is commonly assumed that the observed separations are a result of continuous partitions between the aqueous stationary phase and the water-im-

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miscible mobile phase. This hypothesis evidently does not hold in chromatographic development with water-miscible solvents or aqueous solutions in which only one phase exists[9].

Identification of the individual spots has been effected determining their R_F value (position relative to the solvent front). Reproducibility of R_F values is the crucial aspect of most methods and appears to be most difficult to obtain. They have been found to vary, not only with temperature, with variations of solvent composition and of pH, with the size of the chamber used for development[3], but also with the method of measurement of R_F, which raises the question of locating the real geometric or densitometric center of the spot. Because of these variables, a reference substance is usually run simultaneously with the unknown samples. Some workers have substituted for the R_F value, the ratio of the distance traveled by the individual spots to that of a reference substance. GOLDBAUM AND KAZYAK[4] have used codeine for this purpose. This ratio is considered to be more reproducible than R_F values.

In the actual problem of separating the major alkaloids of opium, the methods previously referred to are more or less satisfactory. More than one system appears to be needed. Under various conditions (propanol–water with KH_2PO_4-treated paper, *n*-butanol–HCl with KCl-treated paper or *n*-butanol–acetic acid with CH_3COONa-treated paper), MUNIER[18] succeeded in resolving from mixtures of pure products, morphine, codeine and thebaine, but not narcotine from papaverine. MANNERING[12] also failed to separate mixtures of pure narcotine and papaverine, either with isoamyl alcohol–acetic acid, isoamyl alcohol–ammonia or *n*-butanol–acetic acid systems on paper buffered with phosphates. Moreover, the last system could not separate codeine from thebaine. Other workers[7,13] encountered the same difficulty when using the butanols as solvents. BELLES *et al.*[2] did not include narcotine in an otherwise extensive survey of alkaloids separated by descending paper chromatography. A more recent method by SCHMALL, WOLLISH AND SHAFER[21] using multibuffered paper, does not resolve narcotine from papaverine. The authors state that compounds having the same p*K* value cannot be separated by their technique.

In all the preceding papers, there is no mention of any attempt to apply the technique to actual opium extracts, although MUNIER[18] claims that preliminary extractions and purifications of vegetal samples would be necessary in order to eliminate pigments, salts and lipids.

The object of the present study was to develop a single procedure which would allow the actual separation of the major opium alkaloids directly from the natural product.

EXPERIMENTAL

I. Preliminary investigation

General procedure

All experiments were performed with the ascending technique, on rectangular sheets of paper fastened into cylinders with staples. Glass jars (or conventional aquariums) covered with a heavy glass plate were used as chambers. The samples were applied in the smallest area feasible and dried with a current of hot air. After development, the sheets were removed, opened flat and dried under the hood with the aid of an electric fan. They were examined first under ultraviolet light. Solvent front and fluorescent zones were noted. The sheets were then sprayed as evenly as possible with potassium iodoplatinate reagent according to MUNIER[18] and dried with a current of air.

Pure alkaloids

The pure alkaloids were used either as free bases or hydrochlorides and sulfates (dissolved in 95% ethanol). The reference solution was made by dissolving 0.50 g of morphine, 0.06 g of codeine, 0.04 g of thebaine, 0.325 g of narcotine and 0.05 g of papaverine, to 100 ml with 95% ethanol, to approximate the proportions encountered in the natural product.

Papers

Whatman No. 1 or 3MM and Schleicher and Schuell No. 576 were found satisfactory. Development with Whatman No. 4, a less retentive paper, was more rapid, but the spots had a tendency to be somewhat more diffuse.

Pretreatment of paper

The papers were used either as such or were treated with various salts and buffers. These included a wide range of concentrations of chlorides, sulfates, phosphates and acetates of monovalent cations (potassium, sodium and ammonium). Phosphate–citrate, acid phosphate and barbiturate buffers were also investigated.

The most satisfactory procedure for the treatment of paper is described in Part II.

Mobile solvents

Both *n*- and isobutanol solvents were found satisfactory. With the latter, however, it was easier to detect the individual alkaloids present in opium extracts. Amyl alcohols yielded poor results in all the systems investigated. Cyclohexanol was found to behave somewhat like the butanols.

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However, no advantage was found in its use, since it failed to separate narcotine from papaverine. Investigation of other solvents was directed towards the separation of these alkaloids especially and is discussed below.

Acidification of the solvents with acids other than acetic, such as formic, and hydrochloric, was abandoned after various trials. With the latter particularly, there was often formation of more than one liquid front, a phenomenon known as "demixion"[9,18].

TABLE I

R_F VALUES AND CODEINE RATIOS OF ALKALOIDS

Developed with isobutanol–acetic acid 5:1 solvent and 2% $(NH_4)_2SO_4$-treated paper.

	Mixture of pure alkaloids	*Opium extracts*
	R_F value	
Morphine	0.27–0.30 (0.29)*	0.29–0.30 (0.29)
Codeine	0.40–0.45 (0.43)	0.45–0.54 (0.49)
Cotarnine	0.58–0.68 (0.62)	— —
Thebaine	0.76–0.84 (0.79_5)	0.75–0.83 (0.78)
Narc. Papav.	0.87–0.93 (0.89)	0.84–0.91 (0.87_5)
	Codeine ratio	
Morphine	0.66–0.70 (0.68)	0.56–0.65 (0.60)
Codeine	— (1.00)	— (1.00)
Cotarnine	1.37–1.51 (1.44)	— —
Thebaine	1.81–1.90 (1.86)	1.54–1.76 (1.60)
Narc. Papav.	2.04–2.18 (2.09)	1.69–2.00 (1.79_5)

* Average values in parentheses.

TABLE II

R_F VALUES AND CODEINE RATIOS OF ALKALOIDS

Developed with water-saturated *n*- or isobutanol in identical conditions.

	2% $(NH_4)_2SO_4$-treated paper		*M/2 KH_2PO_4-treated paper*	
	Water-satd. n-butanol	*Isobutanol*	*Water-satd. n-butanol*	*Isobutanol*
	R_F value			
Morphine	0.15–0.20 (0.18)*	0.13–0.14 (0.14)	0.21–0.24 (0.22)	0.15–0.17 (0.16)
Codeine	0.23–0.29 (0.26)	0.20–0.22 (0.21)	0.28–0.31 (0.30)	0.21–0.25 (0.23)
Cotarnine	0.32–0.39 (0.36)	0.29–0.32 (0.30_5)	0.35–0.38 (0.37)	0.27–0.32 (0.30)
Thebaine	0.56–0.66 (0.61)	0.53–0.60 (0.56_5)	0.56–0.68 (0.62)	0.53–0.61 (0.58)
Narc. Papav.	0.85–0.91 (0.87)	0.91–0.93 (0.92)	0.81–0.92_5 (0.88)	0.93–0.95 (0.94)
	Codeine ratio			
Morphine	0.65–0.73 (0.69)	0.64–0.65 (0.64_5)	0.73–0.77 (0.75)	0.67–0.71 (0.69)
Codeine	— — (1.00)	— — (1.00)	— — (1.00)	— — (1.00)
Cotarnine	1.34–1.39 (1.37)	1.45 — (1.45)	1.23–1.25 (1.24)	1.28–1.29 (1.28)
Thebaine	2.28–2.44 (2.34)	2.65–2.72 (2.68_5)	2.00–2.19 (2.07)	2.44–2.52 (2.47)
Narc. Papav.	2.96–3.96 (3.40)	4.14–4.65 (4.29_5)	2.89–3.08 (2.96)	3.78–4.43 (4.06)

* Average values in parentheses.

The following systems, in order of importance, gave the best results (wide distribution and sharpness of spots):

(a) isobutanol–acetic acid 5:1, paper treated with 2% ammonium sulfate,
(b) *n*-butanol–acetic acid 5:1, paper treated with 2% ammonium sulfate, and
(c) *n*-butanol–acetic acid 5:1, paper treated with *M*/2 KH_2PO_4.

Freshly prepared solvents were used throughout the study to avoid formation of increased amounts of butyl esters.

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Aqueous saturation of paper and chamber humidity

Exact control of the degree of humidity of the paper during development was one crucial aspect of the methods investigated. Failure to respect this usually ended in poor resolution, often in trailing of the spots. In some cases, the alkaloids, especially those with high R_F values, had a tendency to follow the solvent front. It was also found that, with proper humidity, the rise of the organic phase was more uniform.

Several means of controlling chamber humidity were investigated. Solvent-saturated water or solvent-saturated acid solutions gave equally poor results with isobutanol, although the former was satisfactory for *n*-butanol. Again best conditions of chamber equilibrium are described in Part II.

Identification of alkaloids

Cotarnine, papaverine and narcotine are easily detected by their fluorescence under ultraviolet light. It must be kept in mind, however, that the presence of salts may quench the fluorescence to a high degree and interfere with quantitative estimations by fluorimetry. When they are mixed together, the fluorescence of narcotine is masked by that of papaverine.

Detection of the spots can also be effected by spraying the paper or immersing it into staining solutions. The following reagents were investigated: iodine–iodide[12,18], Dragendorff's reagent[12], thallium–iodine[12,18], bromphenol blue[10], ceric sulfate[22] and potassium iodoplatinate. The latter, developed by MUNIER[18], was found the most convenient indicator reagent in the present study. It has the advantages of giving various colorations with different alkaloids and of being highly sensitive. MANNERING[12] claims that it can detect 2 μg of morphine, codeine or thebaine, 3 μg of papaverine and 5 μg of narcotine, figures in accord with those obtained in the present investigation.

R_F values and alkaloid ratios

Height reached by the solvent front, in a given time, was found to vary with the size of the chamber. This affected R_F values. Uncontrolled variations of pH of buffered paper also altered R_F values. Slight variations of temperature (18–23°) had a less marked effect. In general, R_F values calculated from the densitometric center of the spots, rather than from the geometric center, were more reproducible. This aspect of the problem suggests further investigation. Although attempts to substitute alkaloid ratios for R_F values were somewhat more successful (Tables I and II), it was found more convenient, in the last resort, to run a reference solution of mixed alkaloids along with the unknown samples.

II. Chromatography of opium

1. *Preparation of extract*

Samples weighing 0.5 g were thoroughly ground with 5 ml of a water–glacial acetic acid mixture 1:1 and centrifuged. The supernatant liquid was used directly for chromatographic development.

2. *Buffered paper*

Whatman No. 1 paper (28 cm wide, 32 cm high) was satisfactory. The sheets were immersed in a 2% solution of ammonium sulfate (or *M*/2 potassium dihydrogen phosphate) in water. The excess of liquid was removed first by draining then by sponging the sheets lightly between layers of towel paper. The sheets were allowed to dry vertically, at room temperature, with the aid of a fan.

3. *Developing solvent*

A mixture of 100 parts of isobutanol (reagent grade) and 20 parts of glacial acetic acid was saturated by adding water dropwise (about 36–40 parts) with constant agitation (electric or magnetic stirrer) until turbidity persisted for not more than 45–60 seconds. A volume of 50 ml of freshly prepared solvent was required for each sheet to be

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chromatographed. The solvent was recuperated and used in subsequent experiments for saturating the chamber atmosphere before and during development.

4. *Chromatographic procedure*

After the samples had been applied, the sheets were left for at least one hour to reach phase equilibrium. For this purpose, two sheets of filter paper, fashioned in the same

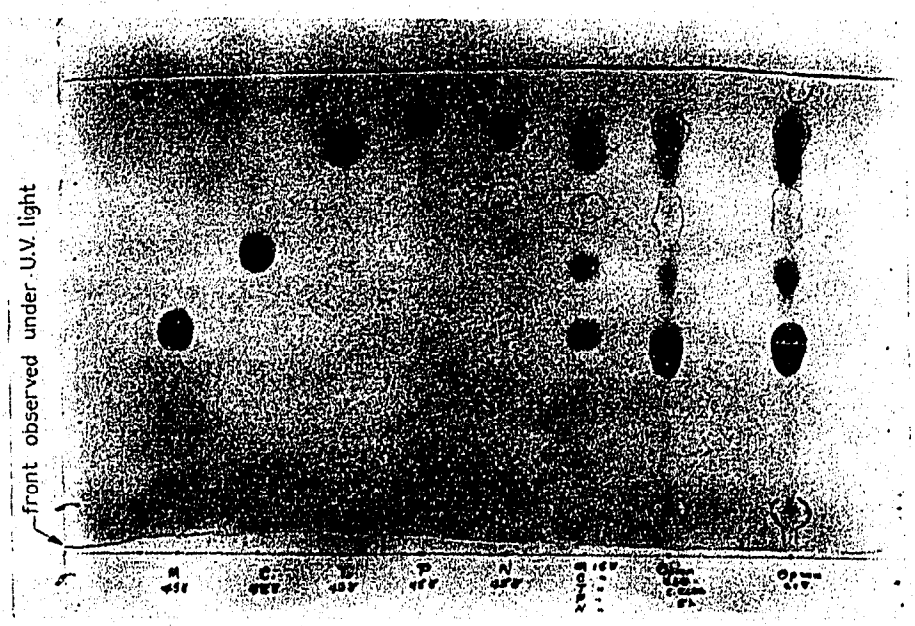

Fig. 1. Chromatogram of pure samples of alkaloids (individually and collectively), along with two opium extracts (after K iodoplatinate spray).

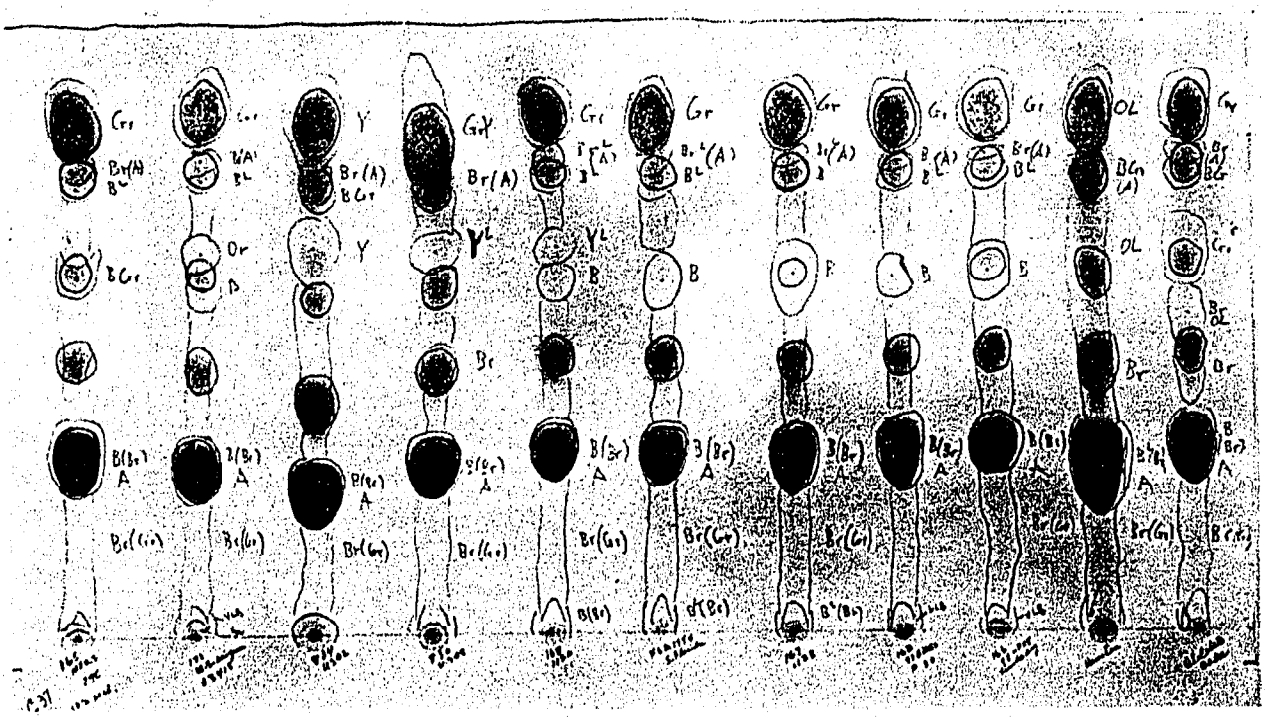

Fig. 2. Chromatogram of opium samples from various sources (origin) after K iodoplatina spray. The fluorescent zones have been circled.

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manner, were wetted and placed, one in a dish containing water and the other in a dish containing recuperated solvent. These sheets were left in the chamber during development. This procedure allowed rapid saturation of atmosphere.

The sheets were then lifted and 50 ml of solvent were poured into the dish. The solvent was allowed to rise to a definite level near the upper edge regardless of the time required to reach it. This precaution was not necessary when the reference solution was run simultaneously on each sheet. This step took from 16 to 17 hours depending upon temperature (18°–23°) and size of chamber. With Whatman No. 4 paper, the time was reduced to 6–7 hours.

Results and comments

Cotarnine, narcotine and papaverine were easily detected under ultraviolet light in the present method. Fluorescence of papaverine masked that of narcotine, when the alkaloids were together. Potassium iodoplatinate reagent stained morphine in deep blue, the other alkaloids in various shades of violet. The spots had a tendency to fade on long standing, but they were revealed again by spraying with the reagent. Trace alkaloids and other constituents of opium did not interfere when solutions obtained by direct extraction with acetic acid were used (Figs. 1 and 2).

Elongation or trailing appeared unavoidable when concentrations were high (Fig. 1). Occasionally the solvent failed to reach the expected level. Under these

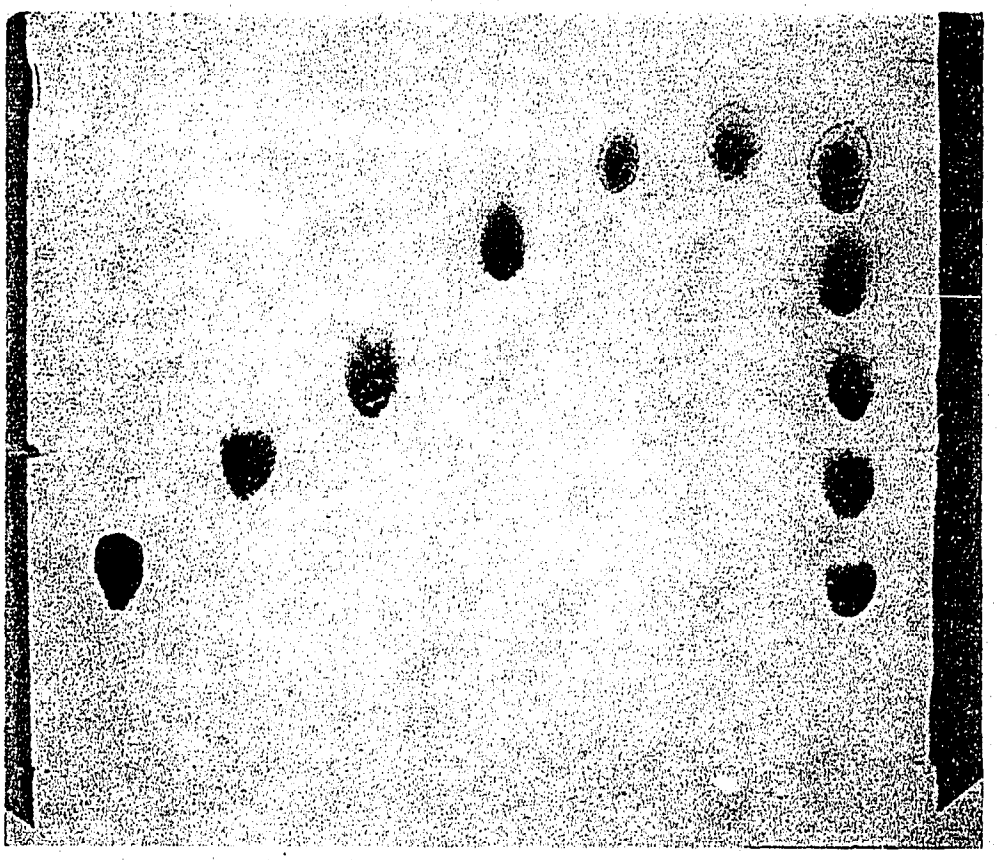

Fig. 3. Chromatogram of (from left to right) morphine, codeine, cotarnine, thebaine, narcotine and papaverine and a mixture of the five alkaloids.

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circumstances, it was observed that the alkaloids with high R_F values had coalesced just below the solvent front forming spots with a flattened appearance. Some alteration in the equilibrium between solvent and aqueous phase during development appears to be involved. For instance, this difficulty arose with certain batches of paper (differences in water adsorption?) and also when an excess of water had been added during the preparation of the solvent.

No differences in R_F values were noted between bases or salts (hydrochlorides, sulfates or acetates in case of extracts). The alkaloids were distributed in the following order of increasing R_F value: morphine, codeine, cotarnine, thebaine and finally narcotine along with papaverine (Tables I and II and Fig. 3).

III. Separation of narcotine and papaverine

None of the solvents which have been described in this communication for the separation of the major opium alkaloids are effective in separating narcotine from papaverine.

In a recent publication, KROGERUS[7] reports that the use of acidified dioxan or acidified ether as mobile solvents will result in the separation of narcotine from papaverine on untreated Whatman No. 1 paper. Repeated trials with various lots of dioxan have failed in this laboratory to yield the results obtained by the author. However, the ether solvent has been applied successfully to two-dimensional chromatography in order to achieve the separation of these two alkaloids following their separation from the other major alkaloids. In the first solvent, morphine, codeine and thebaine were separated from narcotine and papaverine. The paper was dried, rotated at a 90° angle and then inserted into the second solvent, ether saturated with aqueous phase, in which the desired separation of narcotine from papaverine is obtained.

Materials

The equipment and materials employed was the same as those previously described (Part II).

Preparation of ether solvents

The solvent suggested by KROGERUS[7] was used. This was prepared by shaking 100 ml of ethyl ether with 40 ml of 0.1 *M* acetic acid. The aqueous phase was used to saturate the atmosphere of the developing chamber while the organic phase was employed as the mobile solvent. Two other solvents were prepared by substituting for the 40 ml of 0.1 *M* acetic acid, the same volume of water or of 0.2 *M* acetic acid. The results obtained with the last, however, were not significantly different from those obtained with 0.1 *M* acetic acid and therefore are not considered in this report.

Presaturation of papers

If the chromatograms, after treatment with the first solvent, were placed immediately in the ether solvent without a presaturation period, some narcotine and/or papaverine could always be detected at that spot where they had been deposited by the first

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solvent. This difficulty was obviated by allowing a three hour presaturation period in the presence of aqueous phase before the addition of the mobile solvent. When narcotine and papaverine were spotted on fresh, untreated Whatman No. 1 paper or on paper pretreated with salt, however, quantitative removal of the alkaloids from the baseline was never obtained despite the long presaturation period.

Procedure

For two-dimensional chromatography, the material under study was spotted at two points, each located about 2.5 cm from the bottom and 2.5 cm from either edge of an appropriately treated sheet of Whatman No. 1 paper (28 cm × 32 cm).

Development in the first solvent was permitted to take place according to the method previously outlined. After its removal from this solvent, the sheet was dried and examined under ultraviolet light so that the solvent front and fluorescent zones (particularly due to narcotine and papaverine) might be noted. A strip, 4.5 cm wide, was then cut off the right hand side of the sheet. This strip was sprayed with the indicator reagent in order to determine the number of components separated by the solvent.

The other unstained portion of the sheet was now rotated at a 90° angle, rolled into a cylinder and its edges stapled. The paper was placed in the chamber which also contained a second paper cylinder soaked with aqueous phase and left to saturate in this atmosphere for 3 hours. After this time, 90 ml of ether saturated with aqueous phase (water or 0.1 *M* acetic acid) were used for development. The ascent of the solvent to within 1–2 cm of the top edge of the cylinder was usually effected in about 2 hours. The sheet was then removed, dried, examined under ultraviolet light and stained.

Results

Several combinations of salt-treated papers and mobile organic solvents were shown in a previous section to give clear-cut separations of morphine, codeine and thebaine from one another and from narcotine and papaverine. The best combinations were

TABLE III

THE EFFECT OF TWO ETHER SOLVENTS ON THE SEPARATION OF NARCOTINE AND PAPAVERINE IN TWO-DIMENSIONAL CHROMATOGRAPHY

	Pretreatment	*First solvent*	*Second solvent*	*Results*
1	*M*/2 KH_2PO_4	H_2O-satd. *n*-butanol	Acid*-satd. ether	Separations of narcotine and papaverine are always obtained but there is a tendency for the spots to be diffuse and irregular in appearance
2	*M*/2 KH_2PO_4	H_2O-satd. *n*-butanol	H_2O-satd. ether	
3	2% $(NH_4)_2SO_4$	H_2O-satd. *n*-butanol	Acid*-satd. ether	
4	2% $(NH_4)_2SO_4$	H_2O-satd. *n*-butanol	H_2O-satd. ether	
5	2% $(NH_4)_2SO_4$	Isobutanol–acetic acid 5:1	Acid*-satd. ether	
6	2% $(NH_4)_2SO_4$	Isobutanol–acetic acid 5:1	H_2O-satd. ether	Narcotine and papaverine separate. Best conditions insofar as the final appearance of the spots is concerned

* 0.1 *M* acetic acid

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selected in an attempt to determine with which one(s) could be obtained the most satisfactory separation of narcotine and papaverine in a second solvent. Both water-saturated ether and ether saturated with 0.1 *M* acetic acid were tried as second solvents. The results of these experiments are summarized in Table III. It will be noted that narcotine and papaverine were always separated by either of the ether solvents used. The position of the other alkaloids was not disturbed by the run in the second solvent under the conditions listed in 1, 3, 4 and 5 (Table III), but thebaine migrated a short distance under the conditions listed in 2 and codeine and thebaine under the conditions in 6. This, however, had no effect on the final interpretation of the results. In fact, insofar as the chromatography of opium extracts is concerned, the displacement of codeine and thebaine in the second solvent provides a convenient check in cases where their presence in low concentrations may have rendered uncertain their identification after separation in the first solvent.

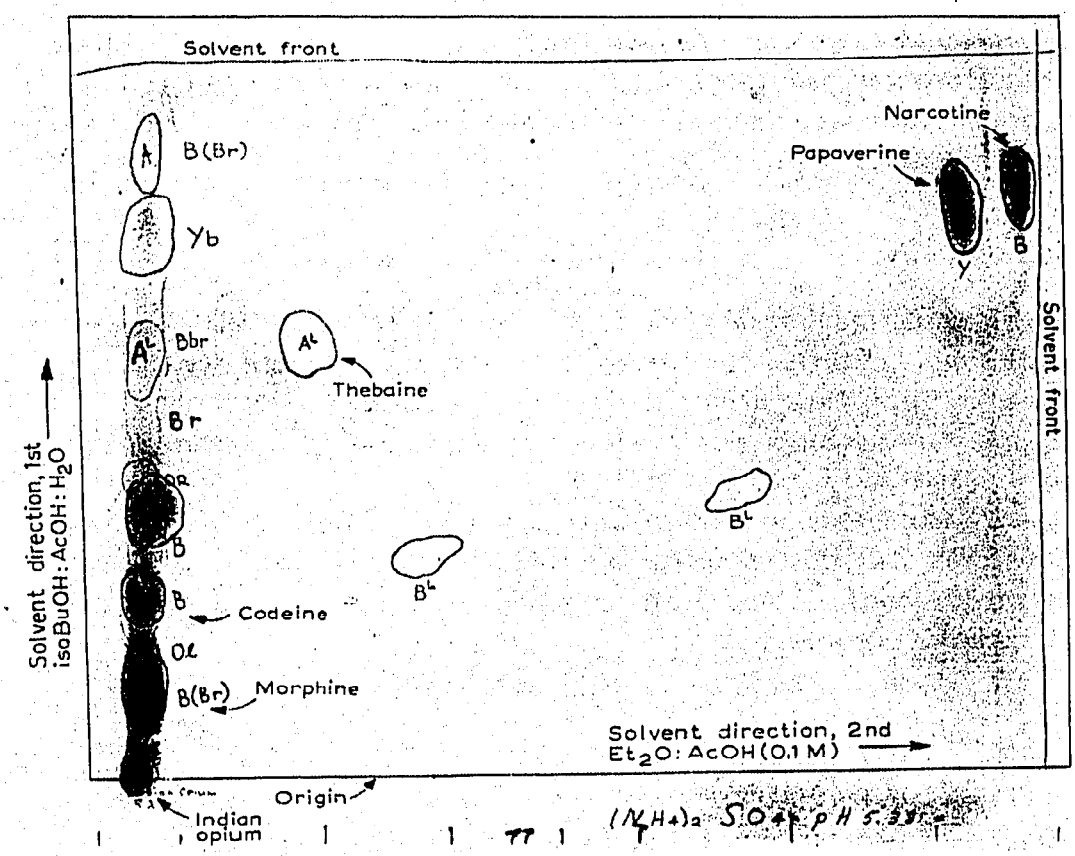

Fig. 4. Two-dimensional chromatogram of indian opium (export) in isobutanol–acetic acid and ether saturated with acetic acid (0.1 *M*) showing separation of papaverine and narcotine from morphine, codeine and thebaine.

The tendency for the alkaloids to streak or to be deposited over large ill-defined zones by the second solvent was the major drawback to most of the conditions studied. Of all the conditions tested, the best results were obtained by using paper treated with 2% $(NH_4)_2SO_4$, isobutanol–acetic acid 5:1 as the first solvent, and water-saturated ether as the second solvent (No. 6, Table III). Even so, the final narcotine and papa-

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verine spots were usually elliptical rather than round in shape, but the separation was clear-cut and there was no evidence of streaking (Fig. 4).

A considerable variation in the R_F values of narcotine and papaverine was obtained. In six experiments performed under the aforementioned conditions (Table IV), the R_F values of narcotine ranged from 0.72 to 0.91 and those of papaverine from 0.62 to 0.78. However, the ratio of the distances traveled by each alkaloid was fairly constant. In the same six experiments, the narcotine:papaverine ratio varied between 1.13–1.21 with a mean value of 1.16.

TABLE IV

R_F VALUES OF ALKALOIDS DEVELOPED WITH WATER-SATURATED ETHER IN SECOND DIMENSION
First dimension: isobutanol–acetic acid 5:1 on 2% $(NH_4)_2SO_4$-treated paper

Morphine	0.0	0.0	0.0	0.0	0.0	0.0
Codeine	0.16	0.13	0.03	0.05	0.12_5	0.14
Thebaine	0.31_5	0.34	0.13	0.21	0.36	0.27
Papaverine	0.78	0.78	0.73_5	0.64	0.73	0.62
Narcotine	0.91	0.89	0.88	0.72	0.82	0.75
N/P ratio	1.17	1.14	1.20	1.13	1.13	1.21

CONCLUSIONS

Following the investigation of various parameters involved in the paper chromatography of alkaloids, alternative methods are presented here for the separation and identification of important alkaloids in opium (namely morphine, codeine, thebaine, narcotine and papaverine), using an acetic acid extract obtained directly without further purification.

It appears from the present investigation and from all the available data that alcoholic systems will not separate narcotine from papaverine. However, by using these systems in conjunction with another in a two-dimensional procedure, this difficulty can be overcome.

Addendum

Since this paper was read (Annual meeting of the Forensic Society of Canada, October 1956), the thesis by BETTSCHART[25,26] has been brought to our attention. The work constitutes a thorough survey of various parameters involved in the chromatographic separation of tropine and opium alkaloids. On a more practical point of view, the author has used with advantage a butanol solvent associated with a phosphate–citrate buffer (pH 6.8) by descending paper chromatography for the separation of mixtures of pure morphine, codeine, narceine and thebaine. It is claimed that narcotine and papaverine can only be separated at a pH between 3 and 4.5, using ether as solvent. This is not in accord with our findings. The author does not suggest a definite technique for the separation of the five major alkaloids of opium in a single procedure. Buffered paper at two different pH values being required, a two-dimensional procedure is

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hardly feasible. The paper-chromatographic method has not been applied to the natural product.

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SUMMARY

Alternative methods are presented for the separation, by ascending paper chromatography, and the indentification of morphine, codeine, thebaine, narcotine and papaverine from crude extracts of raw opium and from mixtures of pure alkaloids. The influence of various parameters involved in the procedure is discussed. Although alcoholic systems will not separate narcotine from papaverine, this difficulty can be overcome by using acidified or aqueous ether as second solvent in a two-dimensional procedure.

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