Opium Alkaloids—Separation and Identification by Gas, Thin-Layer, and Paper Chromatography

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A large number of opium alkaloids has been chromatographed by gas, thin-layer, and paper chromatography for the purpose of identification. Gas chromatography is particularly valuable because of its speed, simplicity, and reproducibility. The two other methods are useful for certain alkaloids which either do not lend themselves to gas chromatography or give unsatisfactory separation.

FEW NATURAL products have been studied so extensively as opium, and none has presented problems of such magnitude from chemical, biological, and social viewpoints. The principal alkaloids of opium were discovered during the first half of the 19th century. During the next 50 years, many minor alkaloid constituents were isolated, and the list has continued to grow even during the 20th century. Today perhaps 20 are recognized as genuine alkaloids in opium and the opium poppy. In addition, several heterocyclic nitrogen compounds have been isolated which probably do not occur in the fresh latex but are formed as a result of oxidation, hydrolysis, or racemization.

Most of the opium alkaloids which have been isolated as pure compounds are now reasonably well known and, except for a few, their structures have been established. Nevertheless, it is not an easy task to identify the minor alkaloids of opium because they are often difficult to purify, and the identity tests which have been described are not specific. Thus, Fulton (1) has reported that he has isolated several phenolic opium alkaloids which he has been unable to identify. Recently, Pfeifer and Teige (2) have described a new opium alkaloid whose structure has not yet been established. More alkaloids will undoubtedly be discovered as modern methods of isolation and characterization are developed. In connection with such work, it is essential to be able to identify microquantities of the alkaloids which have already been isolated. For this purpose, the chromatographic behavior of a large number of opium alkaloids was studied by gas, thin-layer, and paper chromatography.

EXPERIMENTAL

Gas Chromatography.—The instrument used for this work was a Barber-Colman model 15 gas chromatograph equipped with an argon ionization detector and U-shaped glass columns 6 ft. long and $^1/_8$ in. in inside diameter. The solid support was Gas-Chrom A, washed with acid and base and siliconized with hexamethyldisilazane (3). It was inactivated further with thin coats of polyethylene glycol 4000 and nonylphenoxypolyethyleneoxyethanol, each coat representive of about 0.05% of the weight of the support. This material was coated rapidly with 2% of silicone rubber SE-30 by a filtration method (4). The alkaloid samples were dissolved in acetone and applied with a microsyringe as 1 μl. containing 5 to 10 mcg. of each alkaloid. The results are given in Table I.

Thin-Layer Chromatography.—Silica gel G was used as the adsorbant, and the chromatograms were developed with methanol–chloroform (1:9) and ethanol–benzene (1:4), as described by Mary and Brochmann-Hanssen (5). After drying, the plates were observed under ultraviolet light and sprayed with potassium iodoplatinate reagent. In the case of porphyroxine, the plate was sprayed with hydrochloric acid and dried; a characteristic red spot was produced. Occasionally, spraying with Gibbs' reagent (2,6-dichloroquinone chlorimide) was used to develop the spots of laudanine, laudanidine, and reticuline. The R_f values in thin-layer chromatography greatly depend on the brand of silica gel and the activation of the thin-layer plates. The results in Table II are average values based on a large number of runs.

Paper Chromatography.—Of the many solvent systems used for paper chromatography of alkaloids, a modification of the procedure described by Miram and Pfeifer (7) appeared to be most satisfactory for the opium alkaloids. Sheets of Whatman No. 1 filter paper were cut in the machine direction into strips 46 cm. long and 20 cm. wide. With a soft lead pencil, lines were drawn across the papers 6.5 cm. and 32.0 cm. from one end; the first line marked the starting point. The papers were sprayed with

TABLE I.—RELATIVE RETENTION TIMES OF OPIUM ALKALOIDS

Alkaloid	
Codeine	0.38
Neopine	0.41
Morphine	0.48
Thebaine	0.59
10-Hydroxycodeine	0.66
Laudanosine	1.00
Laudanine	1.12
Laudanidine	1.12
(±)-Reticuline	1.18
Papaverine	1.65
Protopine	1.95
Cryptopine	2.41
Narcotine (noscapine)	4.40
Carrier gas	Argon
Inlet pressure, p.s.i., lb.	19
Column dimensions	6 ft. × $^1/_8$ in.
Liquid phase	2% SE-30
Column temp.	207°
Injection port temp.	302°
Cell bath temp.	230°
Laudanosine time, min.	16.4

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TABLE II.—R_f VALUES OF THIN-LAYER CHROMATOGRAMS

Alkaloid	Solvent System MeOH:CHCl₃ (1:9)	Solvent System EtOH:C₆H₆ (1:4)
Narceine	0.09	0.03
Morphine	0.12	0.08
10-Hydroxycodeine	0.17	0.15
(±)-Reticuline[a]	0.28	0.18
Codeine	0.35	0.15
Neopine	0.38	0.12
Protopine	0.46	0.42
Laudanine	0.47	0.28
Laudanidine	0.47	0.28
Cryptopine	0.48	0.40
Thebaine	0.65	0.38
Laudanosine	0.74	0.36
Narcotoline	0.88	0.71
Porphyroxine[b]	0.93	0.65
Papaverine	0.97	0.77
Narcotine (noscapine)	0.97	0.87

[a] Isolated from opium (6). [b] Isolated from opium as described by Pfeifer and Teige (2).

McIlvain buffers, as illustrated in Fig. 1. The excess of buffer solution was removed between clean filter papers, and the paper strips were allowed to air-dry for 2 hours. Spots of the alkaloid solutions (in chloroform or methanol) were placed on the starting line 3 cm. apart; after a few minutes, the papers were placed in chromatographic jars, the atmosphere of which was saturated with the solvent system to be used. After 1 hour of equilibration, the chromatograms were developed with water-saturated *n*-butanol or water-saturated ether, respectively. The results are illustrated in Fig. 1, a composite picture based on a number of chromatograms.

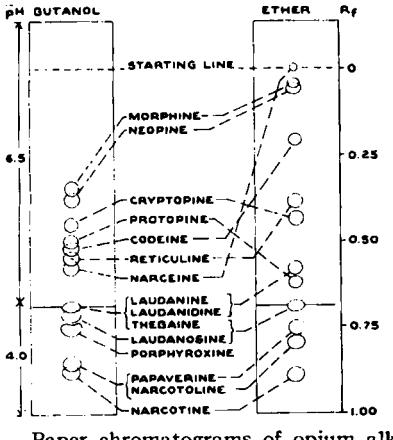

Fig. 1.—Paper chromatograms of opium alkaloids developed by descending chromatography.

DISCUSSION

The gas chromatographic method was most generally applicable and permitted separation of the largest number of alkaloids. Even when no clear-cut separation of mixtures could be obtained, as in the case of codeine and neopine or laudanine and reticuline, there was difference enough in the retention times to make possible the identification of the pure alkaloids. The isomeric alkaloids, laudanidine and laudanine (*dl*-laudanidine), could not be separated by the three methods. The peak shift technique described by Anders and Mannering (8) was very useful for the identification of phenolic alkaloids.

Thin-layer chromatography is also a good method for identification of many opium alkaloids, but the retention data are not nearly so reproducible as for gas chromatography. Although several alkaloids have R_f values too close for separation, it is often possible to identify the spots by observing them under ultraviolet light and by using selective spray reagents. The relative differences in R_f values for the two solvent systems are also useful as means of identification.

The paper chromatographic method showed considerable selectivity in some instances where the other two methods were unsatisfactory. It was the only method which gave a clear separation of codeine and neopine.

It would seem that the three methods when considered together should permit identification of practically all known opium alkaloids. When extracts of opium were subjected to these procedures, the alkaloids chromatographed as the pure compounds could readily be identified. It also became apparent that opium contains several alkaloidal substances in addition to those described in this paper. An investigation is being conducted to isolate and characterize these unknown alkaloids.

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