Quantitative Determination of Morphine in Opium by Gas-Liquid Chromatography

By E. BROCHMANN-HANSSEN and A. BAERHEIM SVENDSEN†

Although morphine may be gas chromatographed as such, its phenolic properties cause adsorption which produces tailing and makes quantitative work impossible. This difficulty was overcome by converting morphine to its trimethylsilyl ether which gave a symmetrical elution peak suitable for quantitative estimation. The alkaloids of opium were extracted and morphine separated from nonphenolic alkaloids by ion-exchange resins. The morphine fraction was allowed to react with hexamethyldisilazane, tetraphenylethylene was added as the internal standard, and the solution was gas chromatographed on a column of silicone rubber SE-30.

DURING THE PAST few years, the gas chromatographic method has become increasingly important for analysis of high molecular weight compounds of biological origin. In 1960 Lloyd, *et al.* (1), gas chromatographed 45 different alkaloids, including the major alkaloids of opium at temperatures slightly above 200°. Eddy, *et al.* (2), used gas chromatography for the determination of the origin of opium based on the peak height ratios of several of the major alkaloids.

When working with alkaloids on low-loaded columns, tailing can often cause considerable difficulties. The support material will contain active spots which will not be sufficiently covered by the stationary liquid to prevent adsorptive effects. Adsorption can be reduced to a great extent if the support material is washed with acid and alkali and treated with dichlorodimethylsilane (3), hexamethyldisilazane (4), and/or a surface-active agent (4–6). Phenolic alkaloids, such as morphine, are difficult to gas chromatograph even on a treated support. If adsorption takes place, it will affect the retention time as well as the height and area of the emerging peak, and quantitative work becomes impossible. This difficulty may be overcome by converting the phenolic hydroxyl groups to esters or ethers. Hexamethyldisilazane is excellently suited for this purpose (7). At room temperature it produces trimethylsilyl ethers in quantitative yield.

Perhaps the most crucial step in a quantitative determination of morphine in opium is the extraction. Strongly acidic cation-exchange resins have been shown to effect rapid and efficient extraction of alkaloidal crude drugs including opium (8–11). The total alkaloids thus extracted may be effectively purified and phenolic and nonphenolic alkaloids separated by means of a strongly basic anion-exchange resin (9, 10). Such purification prior to the gas chromatographic analysis increases the life of the column, the specificity of the method, and simplifies the quantitative evaluations.

During the development of the method described below, two internal standards were used, tetraphenylethylene and laudanosine, which are eluted on either side of morphine 3,6-di-trimethylsilyl ether (Fig. 1). Both standards, however, give the same results. Tetraphenylethylene is readily available and is stable at the experimental conditions of this method. Most of our samples were analyzed with this substance alone as the internal standard.

EXPERIMENTAL

Twelve opium samples were analyzed. Eleven of these were authenticated samples obtained through the United Nations' Division of Narcotic Drugs.

Preparation of the Sample.—Two-hundred milligrams of finely powdered opium was triturated in a glass mortar with 1 ml. of hot water. Gradually, 10 ml. of hot water was added while stirring. The stirring was continued for 5 minutes, and the aqueous extract was decanted into an extraction

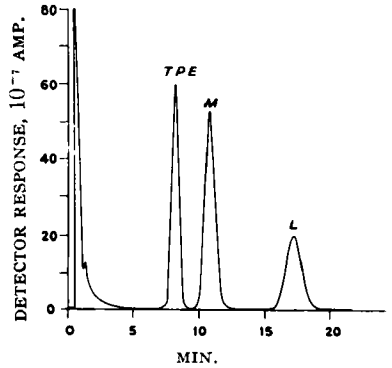

Fig. 1.—Gas chromatogram of morphine (M) isolated from a sample of opium by ion-exchange techniques. Tetraphenylethylene (TPE) and laudanosine (L) are added as internal standards.

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tube (8) containing 3 Gm. of an analytical grade of Dowex 50-X_2, 50–100 mesh. The residue was extracted twice more with 10 ml. of hot water each time and the extracts added to the extraction tube. The opium residue was then washed quantitatively into the tube with enough hot water to give a volume of about 50 ml. The tube was shaken mechanically for 15 minutes. The liquid was allowed to drain slowly from the extraction tube, and the resin in the tube was washed with about 50 ml. of water.

The alkaloids on the resin were eluted slowly with 100 ml. 1 *N* methanolic ammonia (68 ml. of concentrated ammonia, 700 ml. of reagent grade methanol, and enough water to make 1000 ml.). The eluate was allowed to pass through a 1.2 cm. × 30-cm. column of an analytical grade of Dowex 1-X_1, 50–100 mesh. The resin was previously activated with 4 *N* sodium hydroxide and washed free of excess alkali with distilled water. The cationic resin was washed with 50 ml. of 70% methanol, and the washings allowed to flow through the anionic-exchange column. Finally, the anionic-exchange resin was washed with water until the washings were neutral to phenolphthalein, and morphine was eluted slowly with 100 ml. of 0.5 *N* acetic acid. The morphine eluate was collected in a 250-ml. round-bottomed flask and evaporated to dryness in a rotating vacuum evaporator. To remove completely the last traces of water and acetic acid, 10 ml. of absolute ethanol was added and removed under reduced pressure. This was repeated a second time. Thirty-five milligrams of tetraphenylethylene was added to the flask, and the internal standard and alkaloidal residue were dissolved in 2 ml. of pyridine, which had been redistilled over phosphorus pentoxide. One milliliter of hexamethyldisilazane was added to the solution, the flask was stoppered with a glass stopper, and set aside for 24 hours.

Gas Chromatography.—The instrument used for this work was a Barber-Colman model 15 gas chromatograph equipped with an argon ionization detector containing 56 μc. of Ra-226. The column was a glass U-tube, 4 ft. long and 4 mm. in inside diameter. The solid support was Gas-Chrom P, 60–80 mesh, which was washed with concentrated hydrochloric acid, methanolic potassium hydroxide, dried, and treated with hexamethyldisilazane (4). The dried and siliconized support was coated with 0.1% of polyethylene glycol 9000 and, finally, with 4% of silicone rubber, SE-30 (7). The column temperature was maintained at 183°, the injection port (flash heater) temperature was 280°, and the detector cell temperature was 240°. The gas pressure at the inlet was 20 lb., producing a gas flow of 250 ml. per minute. The solutions to be analyzed were injected with a Hamilton microliter syringe, the usual sample size being about 3 μl., corresponding to about 20 to 30 mcg. of morphine. The instrument was operated at submaximal sensitivity to reduce the noise level and gain linearity of detector response. The peak areas were measured with a planimeter, each area reading being based on the average obtained from the cumulative value of ten consecutive tracings. The area ratio of morphine (M) to tetraphenylethylene (TPE) was determined, the corresponding weight ratio obtained from a standard curve, and the percentage of morphine in the sample calculated.

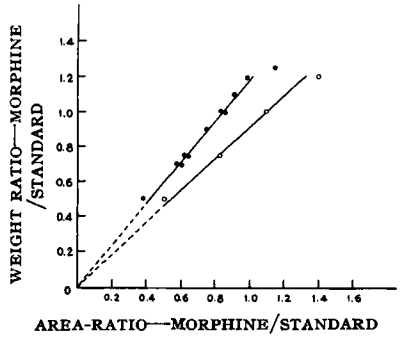

Fig. 2.—Standard curves for morphine with two internal standards. Key: ●, morphine/tetraphenylethylene; ○, morphine/laudanosine.

Standard Curve.—In each of several glass vials were placed 20 mg. of tetraphenylethylene, 20 mg. of laudanosine base,[1] and known amounts of anhydrous morphine base ranging from 10 to 25 mg. The substances were dissolved in 2 ml. of dry pyridine; 1 ml. of hexamethyldisilazane was added. The vials were stoppered with polyethylene stoppers and set aside at room temperature. The reaction appeared to be complete in 16 to 18 hours. An additional reaction time of several days produced no change in the result. The solutions were gas chromatographed, and the weight ratio of morphine to the internal standard was plotted against the corresponding ratio for the peak areas. A straight line was obtained over a range sufficiently great to include all samples encountered (Fig. 2).

The linear range of the argon ionization detector is narrower than that of most other detectors used in gas chromatography (12). However, by adjusting the amount of internal standard added to the unknown it is always possible to work within the linear part of the curve. Although our standard curve appeared to be very reproducible, we deemed it desirable to check it with new standard solutions every day that unknown samples were gas chromatographed.

RESULTS AND DISCUSSION

The contents of morphine in the opium samples analyzed are recorded in Table I. The results are compared with results obtained by other workers using different methods. The reproducibility of the method is satisfactory. Based on a total of 40 determinations of ten different opium samples, the precision, calculated as the standard deviation, was 0.15%.

It is well known that the lime method used by the U.S.P. for determination of morphine in opium does not give results which are absolutely correct. One reason for this is that the crystallization of morphine is incomplete so that a certain amount is lost in the mother liquor (16, 17). It has also been shown that the morphine crystals are contaminated with other opium alkaloids (17). Mannich's method (18), which is based on precipitation of the dinitrophenyl ether of morphine, is considered by many workers to give more accurate results (14, 17, 19, 20).

Fulton (21, 22) has described certain phenolic opium alkaloids which follow morphine very closely

[1] We are indebted to Mallinckrodt Chemical Works, St. Louis, Mo., for supplying us with this alkaloid.

TABLE I.—MORPHINE CONTENT IN OPIUM SAMPLE

Opium Sample	Morphine Content, %—G.L.C. Method[a]—Value Determined	Calcd. to Anhydr. Basis	Other Methods
U.S.P.	11.4	...	10.5[b]
UN2A	14.0	14.9	13.5[c]
UN15	16.5	17.5	16.1[d]
UN38G	18.5	19.7	17.0,[c] 20.3[d]
UN137A	15.1	16.1	13.8[c]
UN25A	18.3	19.5	18.1,[c] 19.07[d]
UNE529	13.8	14.6	13.8[c]
UNE531	11.0	11.7	12.0[c]
UN265	12.8	13.6	13.1[c]
UNE612	15.5	16.3	15.5[c]
UNE627	12.4	13.1	11.0[c]
UNE631	12.2	13.2	12.0[c]

[a] Average values based on two or more determinations. [b] Opium assay U.S.P. XVI (13). [c] Modified Mannich method (14); sample analyzed without drying. [d] United Nations' Secretariat (15); calculated to anhydrous basis.

TABLE II.—GAS CHROMATOGRAPHIC DETERMINATION OF MORPHINE IN OPIUM BEFORE AND AFTER PURIFICATION *via* DICHLOROACETIC ACID

Opium Sample	Morphine, %—No Special Treatment	Purification *via* $CHCl_2COOH$
UN38G	18.6	18.6
	18.4	18.4
UNE265	12.8	12.6
UNE612	15.4	15.4
	15.6	15.5

in many of its reactions and extractions. They are present in amounts up to 1.5% and may lead to high results if the analytical procedure is not sufficiently specific. It is possible to remove them by extracting the acidified morphine fraction with chloroform in the presence of dichloroacetic acid (15). These alkaloids do not interfere in the gas chromatographic determination of morphine. Table II shows that inclusion of an extra purification step *via* dichloroacetic acid does not change the results.

The generally higher results obtained by the gas chromatographic method than by the other procedures referred to in Table I are probably due to a more complete extraction of opium by the ion-exchange resin and a more quantitative recovery during the purification steps.

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2-Aminobenzenethiol Derivatives as Potential Psychotherapeutic Agents

By KARL A. NIEFORTH

A structural similarity between reserpine and chlorpromazine is described and a series of derivatives of 2-aminobenzenethiol designed to match the similarity is synthesized and tested for pharmacologic activity.

THE APPARENT tranquilizing activity of reserpine and chlorpromazine is qualitatively the same, although the two compounds are quite different structurally. It is postulated that the two compounds act by two pharmacologically distinct mechanisms—reserpine by trophotropic stimulation and chlorpromazine by ergotropic inhibition according to the terminology of Hess (1).

With the use of molecular models, a structural similarity may be seen between the two compounds (see Fig. 1). The diagram represents the positional relationships of three atoms in the two compounds and shows the location of an aromatic structure. Position *A* represents the phenolic oxygen of reserpine or the sulfur of chlorpromazine; position *B*, the aromatic nitrogen of each compound; and position *C*, the aliphatic nitrogen of each compound. It must be kept in mind that the positions also could represent any bioisosteric modification. A similarity of

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