β-lipoproteins. A large number of the basic drugs examined resulted in a perturbation of the macromolecule baseline, reflecting that an interaction had taken place. Most of the cationic drugs showing a perturbation had a large hydrophobic portion in the molecule. Perhaps the β-lipoprotein, in order to bind these basic drugs, requires not only the center of deficient electron density but also a hydrophobic area. With the two aliphatic, yet basic, drugs tested, decamethonium and hexamethonium, only a very weak interaction resulted from the larger drug, decamethonium.

The description of the interaction was based on measuring a suitable absorbance difference from at least three titrations and also providing the drug–lipoprotein ratio of such absorbance diferences. In a number of aliquot titrations of the protein, saturation was not reached. In these instances, the results in Table I are the largest absorbance differences recorded at the ratio causing the perturbation.

Considering the results of Refs. 1–3 and those of this preliminary work, it is possible that cationic drugs may be preferentially transported by β-lipoproteins as well as other plasma proteins. If so, then research can be directed toward examining drug–β-lipoprotein interactions when new basic drugs are introduced. The β-lipoprotein can be assessed as to the strength of binding and the numbers of binding sites if some work is done on purification and exact molecular weight determination. Competitive displacement of drugs by concurrently administered agents may be an area in need of further examination if β-lipoproteins have a high affinity for specific basic drugs.

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GLC Determination of Opium Alkaloids in Papaveretum

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Abstract □ A GLC method for the determination of the opium alkaloids in papaveretum, based on the formation of the acetyl derivatives of morphine and codeine, is described. The analytical results are compared with those obtained by the official method. The proposed method is fast and accurate and is particularly suited to the analysis of the raw material.

Keyphrases □ Opium alkaloids—GLC analysis in papaveretum, prepared samples □ Papaveretum—GLC analysis of opium alkaloids, prepared samples □ GLC—analysis, opium alkaloids in papaveretum, prepared samples □ Narcotics—GLC analysis of opium alkaloids in papaveretum, prepared samples

Papaveretum raw material (1) is a synthetic mixture of the hydrochlorides of the opium alkaloids, containing the equivalent of anhydrous morphine (47.5–52.5%), anhydrous codeine (2.5–5.0%), noscapine (16.0–22.0%), and papaverine (2.5–7.0%). It has the analgesic and narcotic properties of morphine but is claimed to produce fewer side effects and may be used in all cases where morphine or opium is indicated (2).

The official method of analysis (1, p. 346), involves a titrimetric method for morphine and gravimetric methods for the other alkaloids. Gravimetric analyses, due to their very nature (3), are prone to error, and the many operations required in the official procedure make it tedious and time consuming. For example, the method involves some 30 extractions, more than 40 washings (*i.e.*, of filter papers and solvent layers), and more than 20 actual transfers of the substances to be determined. A skilled and experienced analyst would require about 2 working days to complete an assay in duplicate.

The GLC determination of the opium alkaloids has been described (4–9). These methods involve initial extraction of the alkaloids, followed by GLC as either the free bases or derivatives. The direct injection of hydrochlorides of various other classes of drugs, which relies on subsequent quantitative breakdown to the free base, was reported (10, 11).

In the present study, a GLC method was investigated involving derivatization of the alkaloid hydrochlorides in papaveretum without prior extraction, followed by direct injection into the gas chromatograph. The acetate derivatives of the hydrochlorides of morphine and codeine are formed, while the hydrochlorides of noscapine and papaverine are not derivatized due to the absence of any free hydroxyl groups. Since no extractions are required, some error-prone steps are eliminated. This study apparently was the first application of this technique specifically to the hydrochlorides of the opium alkaloids in papaveretum.

EXPERIMENTAL

Materials—Standards of morphine, codeine, papaverine, and noscapine bases[1] were used, together with morphine hydrochloride[2]. These materials were dried at 120° for 2 hr immediately before use. Anhydrous analytical reagent grade pyridine was used, and all other solvents were also analytical reagent grade.

A solution of 100 mg of squalane in 100 ml of 95% ethyl acetate and 5% acetic acid was prepared as the internal standard solution.

Equipment and Operating Conditions—The analysis was performed on a gas chromatograph[3] equipped with dual flame-ionization detectors and a 5-mv recorder with a chart speed of 10 mm/min. The columns used were 1120-mm (4-ft) × 3-mm o.d. glass-lined metal tubing[4] packed with

[1] All alkaloid bases were BP grade material, recrystallized before use until they assayed at >99.5% by both GLC and nonaqueous titration.

[2] Customs Department, Sydney, Australia.

[3] Perkin-Elmer F30.

[4] Scientific Glass Engineering, Melbourne, Australia.

Table I—Recovery Results for Papaveretum Alkaloid Bases and Morphine Hydrochloride

Alkaloid	Amount Added, mg	Amount Recovered, mg	Recovery, %
Codeine	54.5	53.2	97.6
	119.1	119.6	100.4
	202.7	201.1	99.2
	216.9	216.7	99.9
			$\bar{x}$ = 99.4
			s = 1.2
Morphine	65.9	63.5	96.4
	128.5	127.9	99.2
	190.7	191.1	100.2
	251.8	251.5	99.9
			$\bar{x}$ = 98.9
			s = 1.7
Papaverine	66.9	65.2	97.5
	120.0	118.9	99.1
	196.9	194.0	98.6
	206.2	207.6	100.7
			$\bar{x}$ = 99.0
			s = 1.3
Noscapine	116.4	117.1	100.6
	191.8	191.5	99.8
	177.4	176.6	99.5
	406.3	401.2	98.9
			$\bar{x}$ = 99.7
			s = 1.3
Morphine hydrochloride	64.2	64.6	100.6
	121.8	120.6	98.9
	192.5	192.7	100.1
	256.5	256.2	99.9
			$\bar{x}$ = 99.9
			s = 0.7

2% OV-101 on 100–120-mesh Gas Chrom Q. The injector and detector were maintained at 300° and the sample was chromatographed under the following conditions: initially at 180° for 5 min, then programmed from 180 to 250° at the rate of 7°/min, and then held at 250° for 3 min. The nitrogen carrier gas flow rate was 30 ml/min, and the air and hydrogen flow rates were adjusted to give optimum detector response. The packed columns were conditioned overnight at 325° with low nitrogen flow (10 ml/min).

Preparation of Standard Solution—*Solution A*—Weigh accurately 200 mg of morphine base and 80 mg of noscapine into a 50-ml volumetric flask. Dilute to volume with anhydrous pyridine.

Solution B—Weigh accurately 80 mg of codeine base and 80 mg of papaverine into a 200-ml volumetric flask. Dilute to volume with anhydrous pyridine.

Table II—Comparison of Chemical (1973 BPC) and GLC Results[a]

Alkaloid	Chemical, % w/w		GLC, % w/w	
	Sample 1			
Codeine	3.83	3.87	3.45	3.25
Morphine	51.66	52.22	51.70	51.54
Papaverine	4.62	4.62	4.19	4.56
Noscapine	20.53	20.70	18.06	18.52
	Sample 2			
Codeine	3.89	3.98	4.05	3.89
Morphine	51.90	51.49	50.93	51.92
Papaverine	4.22	4.33	4.39	4.43
Noscapine	20.03	19.85	18.21	18.98
	Sample 3			
Codeine	2.94		2.53	
Morphine	50.45		50.09	
Papaverine	3.81		3.90	
Noscapine	19.83		19.61	

[a] Duplicate assays.

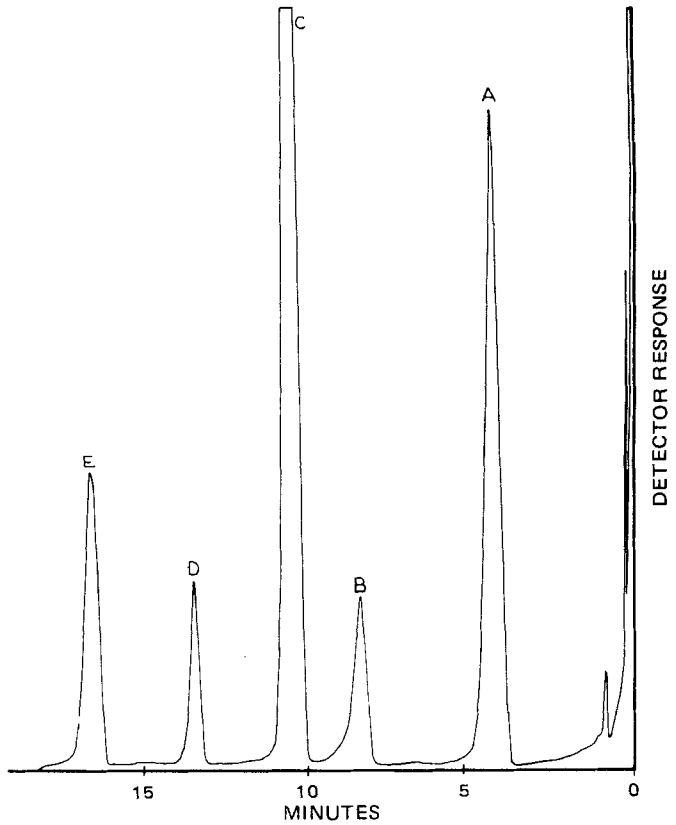

Figure 1—*Typical chromatogram of papaveretum standard preparation. Key: A, squalane; B, codeine; C, morphine; D, papaverine; and E, noscapine.*

Pipet 1.0 ml of Solution A and 1.0 ml of Solution B into a 10-ml conical flask[5] fitted with a septum[6]. Add 1.0 ml of acetic anhydride and allow to stand for 20 min. Evaporate to dryness with a stream of dry nitrogen and add 0.5 ml of internal standard solution. Inject 0.5 μl of this solution into the gas chromatograph, recording the areas of each component using an electronic digital integrator[7].

Preparation of Sample Solution—Accurately weigh approximately 170 mg of the papaveretum sample into a 50-ml volumetric flask, diluting to volume with anhydrous pyridine. Pipet a 2.0-ml aliquot of this solution into a 10-ml flask and then proceed as under *Preparation of Standard Solution* beginning with: "Add 1.0 ml of acetic anhydride . . . ."

The assay is normally performed in duplicate.

Quantitation—Calculate R_{st} and R_s for each component peak from the standard and sample chromatograms, respectively, as follows:

$$R_s = \frac{\text{peak area of particular component in sample chromatogram}}{\text{peak area of internal standard in sample chromatogram}} \quad \text{(Eq. 1)}$$

$$R_{st} = \frac{\text{peak area of particular component in standard chromatogram}}{\text{peak area of internal standard in standard chromatogram}} \quad \text{(Eq. 2)}$$

The amounts of the respective alkaloids in the papaveretum sample are then calculated from the following equation:

$$C_a = \frac{R_s \times C_{st}}{R_{st} \times C_s \times 2} \times 100 \quad \text{(Eq. 3)}$$

where C_a = percent (w/w) of the particular alkaloid base, C_{st} = concentration of that alkaloid in the standard solution in milligrams per milliliter, and C_s = concentration of papaveretum in the sample solution in milligrams per milliliter.

Linearity of Response—The linearity of response of the GLC procedure was checked for each alkaloid using a sample of papaveretum raw

[5] Reacti-Flask, Pierce Chemical Co.
[6] Lined with Teflon (du Pont).
[7] Infotronics CRS-208.

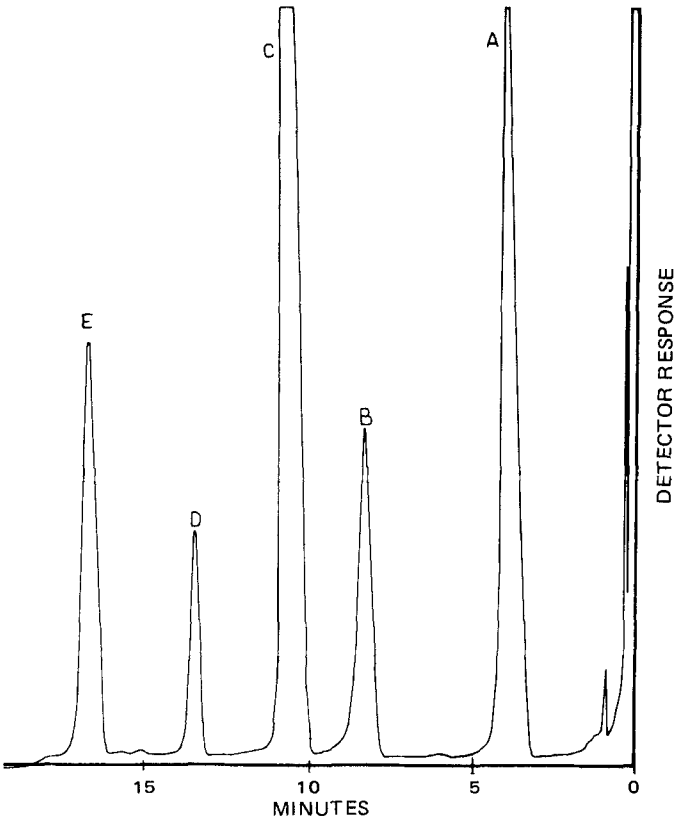

Figure 2—*Typical chromatogram of papaveretum sample preparation. Key: A, squalane; B, codeine; C, morphine; D, papaverine; and E, noscapine.*

material. The response was linear over the 2–20-mg/ml range of papaveretum in the sample solution under the assay conditions described.

RESULTS AND DISCUSSION

Figures 1 and 2 illustrate typical chromatograms of a standard and sample, respectively. The peak due to the acetyl derivative of codeine showed a tendency to tail slightly due to hydrolysis of the derivative to codeine base, brought about by the trace presence of moisture. Normally, the derivatives were stable for at least 4 hr if protected from moisture.

A mixed solvent (95% ethyl acetate and 5% acetic acid) was necessary to keep the derivatives in solution. Ethyl acetate is, by itself, an excellent recrystallizing solvent for diacetylmorphine; therefore, acetic acid was added to increase the solubility of this derivative. The original column has been in use for over 12 months, and no significant deterioration has been observed due to the injection of the acetic acid onto the column.

Recovery experiments were also carried out to check the validity of the method. Table I lists the results for the four alkaloid bases and morphine hydrochloride. Recoveries were quantitative, with an acceptable standard deviation for each alkaloid determination. With the morphine hydrochloride, the results were calculated as morphine base and then factored to the hydrochloride using the theoretical molecular weight ratio. The recovery results obtained in this way verify that the morphine hydrochloride does break down quantitatively to the free base on introduction to the chromatographic system under the conditions described.

Three different commercial lots of papaveretum raw material were assayed in duplicate by both the 1973 BPC method and the proposed GLC method (Table II). Agreement between the two methods was excellent, further validating the applicability of the GLC method.

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Synthesis and Antiparasitic Activity of Certain 2-Imino-3-[(*N*-arylcarbamoyl)methyl]-2,3,4,5-tetrahydrothiazoles

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Abstract □ New derivatives of 2-imino-3-[(*N*-arylcarbamoyl)methyl]-2,3,4,5-tetrahydrothiazole were prepared. Six compounds were tested for their antiparasitic activities and were active in varying degrees against *Hymenolepis diminuta* without any clinical toxicity manifestations.

Keyphrases □ Thiazoles, substituted—synthesized, screened for antiparasitic activity, rats □ Antiparasitic activity—various substituted thiazoles screened □ Structure–activity relationships—substituted thiazoles screened for antiparasitic activity

During the past few years, significant progress has been made in the continuing struggle against parasitic diseases. However, better drugs are still needed for the treatment of many debilitating human and animal parasites. A number of reviews (1, 2) provide background information on the types of chemical structures that possess antipar-