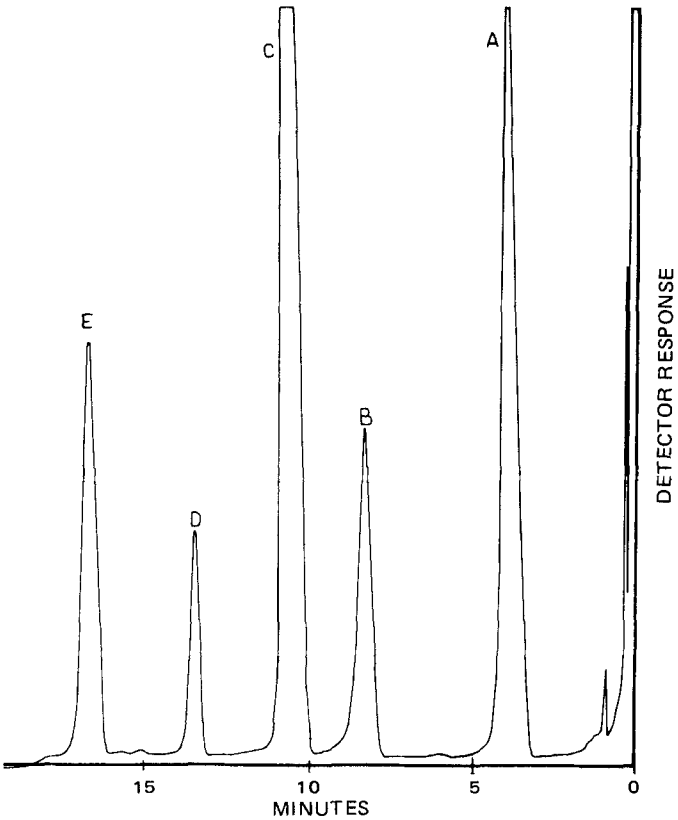

Figure 2—*Typical chromatogram of papaveretum sample preparation. Key: A, squalane; B, codeine; C, morphine; D, papaverine; and E, noscapine.*

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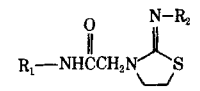
Abstract □ New derivatives of 2-imino-3-[(*N*-arylcarbamoyl)methyl]-2,3,4,5-tetrahydrothiazole were prepared. Six compounds were tested for their antiparasitic activities and were active in varying degrees against *Hymenolepis diminuta* without any clinical toxicity manifestations.

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-

Table I—2-Imino-3-[(*N*-arylcarbamoyl)methyl]-2,3,4,5-tetrahydrothiazoles



Compound	R_1	R_2	Yield, %	Crystallization Solvent	Melting Point	Molecular Formula	Analysis, %	Calc.	Found
I	C_6H_5	H	45	Ethanol–petroleum ether	218–219°	$C_{11}H_{13}N_3OS$ (hydrochloride)	C	48.61	49.21
							H	5.15	4.76
							N	15.46	15.29
II	C_6H_5	$COCH_3$	40	Water	186–187°	$C_{13}H_{15}N_3O_2S$	C	56.31	55.85
							H	5.41	5.42
							N	15.16	14.92
III	$2\text{-}CH_3C_6H_4$	H	43	Ethanol	218–219°	$C_{12}H_{15}N_3OS$ (hydrochloride)	C	50.43	50.31
							H	5.60	5.70
							N	14.71	14.27
IV	$4\text{-}OC_2H_5C_6H_4$	H	60	Hydrochloric acid–water (1:1)	212–213°	$C_{13}H_{17}N_3O_2S$ (hydrochloride)	C	49.44	48.95
							H	5.70	5.78
							N	13.31	12.95
V	$4\text{-}COOC_2H_5C_6H_4$	H	60	Ethanol	209–210° dec.	$C_{14}H_{17}N_3O_2S$ (hydrochloride)	C	48.90	48.77
							H	5.24	5.27
							N	12.22	11.69
VI	$4\text{-}BrC_6H_4$	H	61	Butanol	230–232°	$C_{11}H_{12}BrN_3OS$[a] (base)	C	42.04	43.39
							H	3.82	3.53
							N	—	—
VII	$3\text{-}ClC_6H_4$	H	33	Isopropyl ether	210–211° dec.	$C_{11}H_{12}ClN_3OS$ (hydrochloride)	C	43.13	42.99
							H	3.92	4.18
							N	13.72	13.63
VIII	$4\text{-}NO_2C_6H_4$	H	71	10% Hydrochloric acid	250° dec.	$C_{11}H_{12}N_4O_3S$ (hydrochloride)	C	41.64	41.72
							H	4.10	4.08
							N	17.69	17.29
IX	1-Naphthyl	H	54	Water	230° dec.	$C_{15}H_{15}N_3OS$ (hydrochloride)	C	55.98	56.56
							H	4.97	4.95
							N	—	—

[a] Could not be purified further.

asitic activity.

Recent research on the thiazoles resulted in the development of the leading antiparasitic compounds including nithiamide (3), niridazole (4), thiabendazole (5), and tetramisole (6). Most of these compounds are nitrated thiazole derivatives (3, 4, 7); however, some nonnitrated derivatives are active too (4–6). Certain totally or partially hydrogenated thiazoles (6, 8) are among the active compounds. Antiparasitic nonalkylated thiazoles (4) as well as ring-nitrogen alkylated derivatives (9) also were reported in the literature.

The present paper is concerned with the synthesis and preliminary parasitological testing of new 2-imino-3-[(*N*-arylcarbamoyl)methyl] -2,3,4,5-tetrahydrothiazoles (Table I).

EXPERIMENTAL[1]

2-Aminothiazole—This compound was prepared according to the reported method (10), yielding 39%, mp 76–78° [lit. (10) mp 84–85°].

***N*-Aryl-2-chloroacetamides (X–XVII, Table II)**—These compounds were prepared according to the reported methods.

2-Imino-3-[(*N*-arylcarbamoyl)methyl]-2,3,4,5-tetrahydrothiazoles (I and III–IX, Table I)—To a solution of 2-aminothiazole (0.01 mole) in 10 ml of 2-propanol, 0.01 mole of the appropriate *N*-aryl-2-chloroacetamide was added. The mixture was refluxed for 1.5 hr, and the separated compound was filtered off, washed with cold 2-propanol, and dried. In experiments where no solid separated after cooling, 2-propanol was distilled under reduced pressure and the residue was crystallized from the proper solvent.

2-Acetylimino-3-[(*N*-phenylcarbamoyl)methyl] -2,3,4,5-tetrahydrothiazole (II, Table I)—To a mixture of 2.7 g (0.01 mole) of I and 1.6 g (0.02 mole) of pyridine in 20 ml of chloroform was added 2.04 g (0.02 mole) of acetic anhydride. The mixture was stirred and refluxed for 1.5 hr. After cooling, the mixture was made alkaline with dilute ammonium hydroxide and the organic layer was separated. The aqueous layer was extracted twice with 20 ml of chloroform, and the combined chloroform extract was washed with water and dried (magnesium sulfate). The residue remaining after the distillation of chloroform was crystallized from water.

Materials and Methods of Anthelmintic Testing—The diagnosis of natural infection in laboratory rats was carried out by examination of their stools. Each positive rat was isolated in a separate cage. Positive controls were also isolated in separate cages. A dose of 100 mg/rat (mean weight 100 g) was given orally to a group of rats (two rats were given the same drug). A dose of 20 mg/rat was given in the same way. The stools of all rats were examined daily for *Hymenolepis* ova. Positive rats were redosed and followed up. Negative rats were autopsied to look for the adult worms or scolices. The rat was considered cured when neither ova nor cestodes were recovered.

RESULTS AND DISCUSSION

Chemistry—The new compounds (Table I) were prepared by the interaction of the proper *N*-aryl-α-chloroacetamide (Table II) with 2-aminothiazole (10). Alkylation of 2-aminothiazole by alkyl halides took place at the nuclear nitrogen (19). Compound I was acetylated with acetic anhydride to yield II. The IR spectrum of I showed multiple bands in the 3300–3200-cm^{-1} region (NH stretching), broad multiple bands at

Table II—*N*-Aryl-α-chloroacetamides

$RNHCOCH_2Cl$

Compound	R	Yield, %	Melting Point	Reference
X	C_6H_5	71	137–138°	11
XI	$2\text{-}CH_3C_6H_4$	80	112°	12
XII	$4\text{-}OC_2H_5C_6H_4$	84	142–144°	13
XIII	$4\text{-}COOC_2H_5C_6H_4$	75	116°	14
XIV	$4\text{-}BrC_6H_4$	70	180–181°	15
XV	$3\text{-}ClC_6H_4$	50	101°	16
XVI	$4\text{-}NO_2C_6H_4$	85	185°	17
XVII	1-Naphthyl	56	160°	18

[1] Melting points were determined in open capillary tubes and are uncorrected. IR spectra were determined in potassium bromide using a Beckman IR-20 spectrometer. Elemental microanalysis and IR spectra were performed by El-Nasr Co., A.R. Egypt.

3100–3000 cm^{-1} (secondary amine salt), and strong bands at 1700 and 1570 cm^{-1} (amide, I and II). Compound II revealed broad multiple bands at 3200–3100 cm^{-1} (NH stretching) and strong bands at 1700 (amide-I), 1620 (tertiary amide), and 1540 cm^{-1} (amide-II) (20).

Parasitological Screening—Compounds III, V, and VIII were tested for anthelmintic efficacy in mice naturally infected with *H. diminuta* and were active at the dose level (100 mg/rat, single dose) that showed no activity for VI and VII. Only VIII was active at 20 mg/rat (single dose); a further dose reduction abolished the activity.

Preliminary test revealed the activity of II against *Oxyuris* in naturally infected rats (100 mg/rat, single dose).

All of the drugs tested showed no clinical toxic manifestations even at the highest dose level.

CONCLUSION

The experimental results demonstrate that the replacement of the nitro group in 2-imino-3-[(*N*-4-nitrophenylcarbamoyl)methyl]-2,3,4,5-tetrahydrothiazole (VIII) by ethoxycarbonyl (V) or bromo (VI) substituents results in partial or complete loss of anthelmintic activity. Electronegativity alone cannot explain the crucial role played by the nitro group, and *in vivo* drug bioactivation may be involved.

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Quantitative High-Pressure Liquid Chromatographic Determination of Epinephrine in Pharmaceutical Formulations

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Abstract □ A quantitative high-pressure liquid chromatographic method, using a cationic exchange resin column and an aqueous phosphate buffer as the mobile phase, was employed for the determination of epinephrine in liquid pharmaceutical preparations. The method is stability determining and can differentiate epinephrine in the presence of oxidative and other degradation products.

Keyphrases □ Epinephrine—high-pressure liquid chromatographic analysis, liquid pharmaceutical preparations □ High-pressure liquid chromatography—analysis, epinephrine, liquid pharmaceutical preparations □ Adrenergics—epinephrine, high-pressure liquid chromatographic analysis, liquid pharmaceutical preparations

Epinephrine solutions are widely used for the treatment of open angle glaucoma and are also used as cardiac stimulants and vasoconstrictors. Air oxidation is a major degradation process involved in epinephrine pharmaceutical preparations (1). Antioxidants such as bisulfite are used in many commercial liquid formulations. Bisulfite causes a degradation of epinephrine to form 1-(3,4-dihydroxyphenyl)-2-methylaminoethanesulfonic acid (2).

A stability-indicating method that differentiates epinephrine from its oxidation products as well as sulfonic acid derivatives is needed. The USP XIX assay method requires the formation of the triacetyl epinephrine derivative and is very lengthy (3). Several spectrofluorometric methods have the disadvantage of interference from the antioxidants (4). The addition of antioxidants is necessary to stabilize the final fluorescence in these assay methods.

This report describes a high-pressure liquid chromatographic (HPLC) assay method for epinephrine in liquid pharmaceutical preparations which will differentiate epinephrine from these major degradation products.