using a previous procedure (6) and Table II lists their physical and chemical data.

In agreement with the proposed structures, the NMR data exhibited the common absorption peaks (dimethyl sulfoxide-d_6, tetramethylsilane internal standard): δ 1.10–1.20 (t, 3H, CCH_3), 1.90–2.00 (s, 3H, $COCH_3$), 3.65–3.85 (s, 2H, aryl CH_2C), 4.10–4.20 (q, 2H, OCH_2C), and 6.80–7.30 (m,4H, aromatic) ppm. The spectra of XII and XIII showed a singlet at δ 3.60 ppm that integrated for 5 protons (aryl CH_2 and aryl OCH_3).

Aryl-substituted 5-(1-Amino-2-phenylethyl)tetrazoles XV–XXII)—Compounds XV–XX and XXII were prepared by a previously reported method (6). Compound XXI was prepared by modification of a previously reported method (9). A mixture of compound XX (3.0 g, 0.014 mole) in 48% hydrogen bromide (50 ml) was heated at reflux under nitrogen for 4 hr. The mixture was concentrated under reduced pressure. The residue was dissolved in distilled water and the pH was adjusted to 7 with concentrated ammonium hydroxide. After standing at room temperature for 12 hr, the product was filtered, air dried, and recrystallized from *N,N*-dimethylformamide, 2.2 g (76.6%), mp 290° dec. Table III contains physical and chemical data for XV–XXII.

Assignments of the common NMR absorption peaks are (10% sodium deuteroxide): δ 2.95–3.10 (m, 2H, aryl CH_2), 4.30–4.50 (m, 1H, CCH), and 6.70–7.20 (m, 4H, aromatic) ppm. There was an additional absorption peak for XIX and XX at δ 3.40 (s, 3H, aryl OCH_3) ppm.

Anticancer Screening—The tetrazole analogs of substituted phenylalanines were screened for anticancer activity[2] using P-388 lymphocytic leukemia cells in mice of either sex. On day zero, mice were inoculated intraperitoneally with 10^6 leukemic cells. Twenty-four hours later, a test compound was administered intraperitoneally once daily for the first 9 days or in three injections on every 4th day (XVI, XXIII). The test results were recorded on the 30th day.

[2] Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Silver Spring, MD 20910.

RESULTS AND DISCUSSIONS

All compounds were tested for antileukemic activity at doses of 25, 50, 100, and 200 mg/kg (some in triplicate). For compounds XVI, XVIII, and XIX, the T/C% was near or greater than 125. For all other compounds, the T/C% was < 125 (all mice died when compounds XX and XXIII were administered at 200 mg/kg). Compounds XVI, XVIII, and XIX were retested at 400 mg/kg (in duplicate) and exhibited a T/C% < 125. None of the compounds exhibited significant antileukemic activity.

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Analysis of Commercial Pilocarpine Preparations by High-Performance Liquid Chromatography

MICHAEL V. DRAKE ˣ, JAMES J. O'DONNELL, and ROBERT P. SANDMAN

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Abstract □ Pilocarpine, isopilocarpine, pilocarpic acid, and isopilocarpic acid can be measured effectively by high-performance liquid chromatography (HPLC). Previous reports have differed on the degree of contamination of commercial pilocarpine preparations with isopilocarpine and pilocarpic acid. This report describes a study of commercial pilocarpine in which no significant contamination was found.

Keyphrases □ Pilocarpine—analysis of commercial preparations by high-performance liquid chromatography □ High-performance liquid chromatography—analysis of commercial pilocarpine □ Ocular agents—pilocarpine, analysis by high-performance liquid chromatography

Several assays have been reported for pilocarpine, isopilocarpine, pilocarpic acid, and isopilocarpic acid using high-pressure liquid chromatography (HPLC) (1–4). The earliest of these reports includes the results of a study done on commercial pilocarpine samples, which showed that in one case, contamination with isopilocarpine was as high as 25% (1). An effort to corroborate this study failed, due to technical difficulties (2). Modifications of the original HPLC method were used in a recent study of commercial pilocarpine preparations and no significant contamination with the isopilocarpine isomer or the degradation products, pilocarpic acid or isopilocarpic acid, was found (5). The present report describes a similar study.

EXPERIMENTAL

Each locally available commercial pilocarpine preparation was obtained through prescription from a retail pharmacy. Letters were sent to all other manufacturers[1] of pilocarpine asking for a 1% sample of their product to be used for animal experimentation. Nine fresh samples of pilocarpine were obtained. Each sample was diluted with HPLC grade water to a concentration of 0.10%. Pilocarpine hydrochloride[2] and isopilocarpine hydrochloride[3] standards were obtained commercially in powder form. Pilocarpic acid and isopilocarpic acid were prepared by the hydrolysis of pilocarpine and isopilocarpine in 0.1 *N* NaOH, respectively. These were diluted to make 0.10% standard solutions.

The mobile phase was prepared by dissolving 50 g of monobasic potassium phosphate in a solution of 900 ml of water and 30 ml of methanol. The solution pH was adjusted to 2.5 with 85% phosphoric acid, and the total volume was brought to 1 liter with water. Separation was achieved by isocratic reversed-phase chromatography[4] on an RP-C18 10μm column[5], (flow rate 1.5 ml/min) at ambient temperature. Detection was by optical absorbance at 216 nm[6]. Peak heights of standard preparations were compared with peak heights of the unknown commercial prepara-

[1] Listed in the "Pharmaceutical Drug Topic Redbook" (6) as of January 1979.
[2] Mallinckrodt, St. Louis, Mo.
[3] Aldrich, Milwaukee, Wis.
[4] Model 310 high-performance liquid chromatograph, Altex Scientific, Berkeley, Calif.
[5] Lichrosorb RP-C18 (10 μm) in 4.6 × 250-mm column, Altex Scientific, Berkeley, Calif.
[6] Model 785 variable-wavelength detector, Micromeritics Instrument Corp., Norcross, Va.

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Table I—Commercial Pilocarpine Samples (1%) Compared with a Fresh Pilocarpine Standard

Sample	Percent of Labeled Amount of Pilocarpine Actually Found	Percent Isopilocarpine Found
1	100	1.1
2	99	2.6
3	100	5.5
4	95	5.7
5	101	4.9
6	105	4.0
7	104	1.1
8	102	0.9
9	98	—

tions. There was slight peak tailing; the minimum amount of isopilocarpine detectable in the presence of pilocarpine was 1 part in 100.

RESULTS AND DISCUSSION

Isopilocarpine eluted first at ~10 min followed by pilocarpine, pilocarpic acid, and isopilocarpic acid. The entire process was complete in 20 min. The concentration of pilocarpine found by HPLC analysis was within 5% of the concentration stated by the manufacturer on the label in all cases. The amount of isopilocarpine present ranged from 0.0 to 5.7% of the pilocarpine present (Table I). Pilocarpic acid or isopilocarpic acid were not found in significant quantities in any sample. These results agree with a recent study (5) and are in contrast to another report (1).

There are several possible explanations for the disagreement between this study and the earlier study (1). The official USP method for determining the purity of pilocarpine preparations at the time of the previous report did not effectively distinguish between pilocarpine and isopilocarpine (7). It is possible that pharmaceutical industry standards for purity of pilocarpine preparations have been modified such that they now exceed standards. Another possible explanation is that fresh samples of pilocarpine were used, and degradation products which would have appeared after prolonged storage were not present in these samples.

The results of this study and the study by Noordam *et al.* (5) illustrate the effectiveness of HPLC for pilocarpine assay. Because these newer methods are capable of providing more accurate analysis of pilocarpine than the current official USP method, it is reasonable to consider modifying the USP to reflect more current technology.

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Novel Method of Derivatization of an Amidinourea (Lidamidine) for GLC Analysis

C. M. WON ˣ, J. J. ZALIPSKY, D. M. PATEL, and W. M. GRIM

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Abstract □ A new quantitative GLC method for analysis of lidamidine hydrochloride (I) was developed. The method was based on derivatization of I to 1-(2,6-dimethylphenyl)-4-methylamino-dihydro-1,3,5-triazin-2-one (II) using dimethylformamide dimethylacetal reagent. Compound II was synthesized and characterized by IR, NMR, mass spectrometry, and elemental analysis. The assigned structure was in agreement with characterization analyses. Cyclization of I to a triazinone using dimethylformamide dimethylacetal reagent presented a new route for the preparation of II.

Keyphrases □ Lidamidine—derivatization to a triazinone for GLC analysis □ GLC—derivatization of lidamidine to a triazinone for GLC analysis □ Triazinones—derivatization of lidamidine for GLC analysis

The arylamidinoureas are a family of compounds pharmacologically active on the GI tract, and the cardiovascular and central nervous systems. They also demonstrate local anesthetic activity[1]. Among a series of substituted arylamidinoureas, lidamidine hydrochloride, *N*-(2,6 - dimethylphenyl) - *N'* - [imino(methylamino)methyl]urea hydrochloride (I), exhibited promising antidiarrheal activity. Extensive pharmacological, toxicological, and biochemical studies of I have been reported (1–6). The chemical synthesis (7), the hydrolysis kinetics, and the physical and chemical parameters of I were also reported (8).

Stability-indicating high-performance liquid chromatographic (HPLC) methods[1] were developed to analyze I quantitatively in the presence of impurities or hydrolysis products. A direct GLC method to quantitate I failed because of its low volatility and/or thermal instability. The separation of amidinoureas by the GLC method required derivatization of the functional groups. Derivatization attempts by conventional silylation and acylation methods were unsuccessful.

For the derivatization of primary amines, dimethylformamide dimethylacetal reagent is used to form the *N*-dimethylaminoethylene derivative (9). When substituted guanidines were derivatized by dimethylformamide dimethylacetal, the resultant *N*-dimethylaminoethylene derivative underwent further cyclization reactions to form

[1] William H. Rorer, Inc., internal communication.

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