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

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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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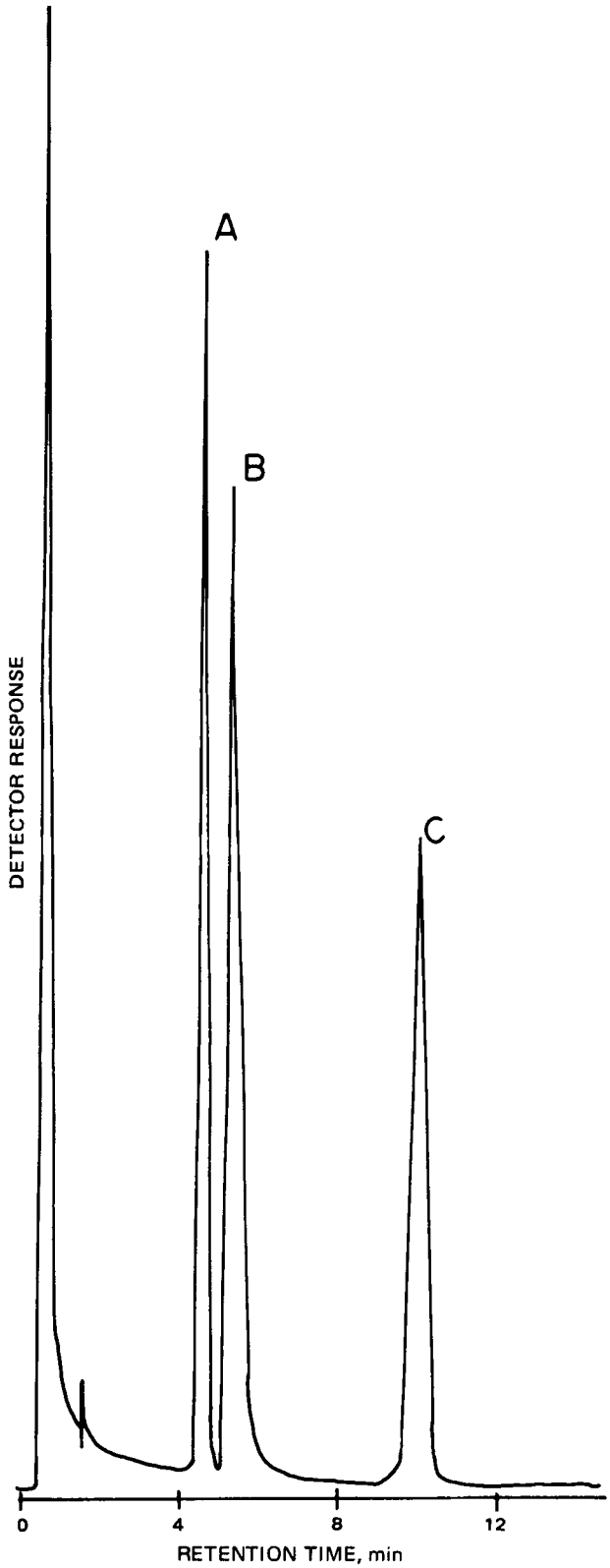


Figure 1—*Chromatogram of internal standard triphenylethylene (A); derivative II (B); and internal standard tetraphenylethylene (C).*

triazine derivatives (10). Biguanides are also known to undergo similar cyclization reactions on derivatization by trifluoroacetic anhydride (11) and *p*-nitrobenzoyl chloride (12).

Dimethylformamide dimethylacetal was used to derivatize I with the hope that a similar reaction would occur and a stable derivative could be formed. The resultant derivative was a triazinone-type compound, which involved cyclization of I during the derivatization.

EXPERIMENTAL

Materials—*N*,*N*-Dimethylformamide dimethylacetal[2], *N*,*N*-dimethylformamide[3], triphenylethylene[4], and tetraphenylethylene[4] were used without further purification.

Instrumentation—A gas chromatograph[5] and an electronic integrator[6] equipped with a flame-ionization detector were used. The 183-cm × 4-mm i.d. glass column was packed with 10% methyl silicone gum[7] on 80–100 mesh diatomaceous earth[8]. The injection port, column, and detector temperatures were maintained isothermally at 270, 250, and 270°, respectively. Nitrogen was used as the carrier gas at a flow rate of 45 ml/min.

Derivatization—Approximately 10 mg of I and 3 mg of an internal standard (triphenylethylene or tetraphenylethylene) were weighed accurately and placed into a 1-ml hypodermic vial and dissolved in 1 ml of *N*,*N*-dimethylformamide. Using a syringe, 0.1 ml of *N*,*N*-dimethylformamide dimethylacetal reagent was added to the vial. The vial was sealed with a silicone rubber septum, shaken, and put in an oven at 110° for 20 min. An aliquot (2 μl) was injected into a gas chromatograph.

Isolation of the Derivative (II)—The derivative was prepared in larger quantity for isolation and characterization. Approximately 200 mg of I was placed in a hypodermic vial and dissolved in 1 ml of acetonitrile. To the solution was added 0.2 ml of *N*,*N*-dimethylformamide dimethylacetal reagent. The vial was sealed and heated at 105° for 15 min in an oven. Seven identical preparations in the separate vials were made. The contents of the vials were pooled and placed in a round-bottom flask and evaporated to dryness under reduced pressure. The resultant solid residue was dissolved in a mixture of 30 ml of chloroform and 20 ml of water in a 60-ml separator and shaken vigorously. The aqueous layer was discarded and the chloroform layer was washed with another 20 ml of water. To the chloroform solution was added 10 g of anhydrous sodium sulfate. The solution was swirled, decanted into a flask, and evaporated to dryness. The white solid residue was recrystallized from 2-pentanone and dried in a desiccator over phosphorus pentoxide under vacuum for 1 hr.

Characterization of the Derivative (II)—The IR spectrum of a potassium bromide dispersion of II was obtained using an IR spectrophotometer[9]. The NMR spectrum[10] was obtained in deuterated chloroform solution with tetramethylsilane as an internal standard. The chemical-ionization mass spectrum[11] was obtained with methane as a reagent gas.

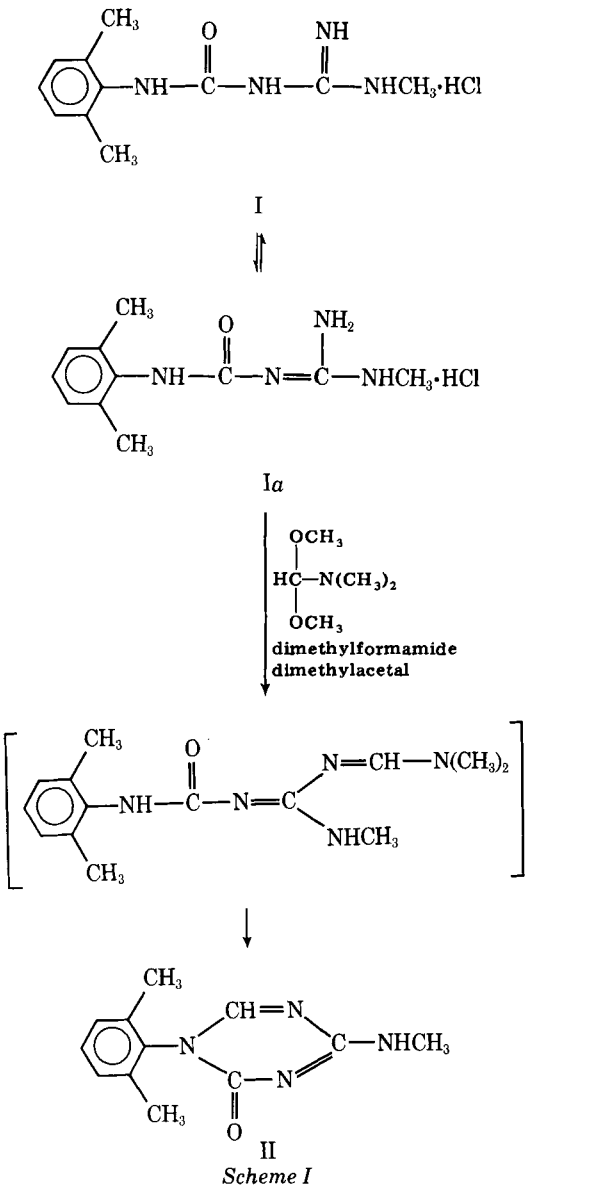
RESULTS AND DISCUSSION

Figure 1 shows a typical chromatogram of II and the internal standards. To establish optimum derivatization time and temperature, a sample containing I and triphenylethylene, an internal standard, was mixed with *N*,*N*-dimethylformamide dimethylacetal and the peak area ratio of II to the internal standard was followed for 3 hr at room temperature. However, the derivatization was not complete during this time period at room temperature. The same experiment at an elevated temperature (110°) showed that the derivatization was complete within 16 min. Dimethylformamide was chosen as the solvent because it is a relatively good solvent for both I and triphenylethylene or tetraphenylethylene and has a high boiling point.

To evaluate the response linearity of the GLC process, I was derivatized at various concentrations (0.9, 1.8, 2.7, and 3.6 mg) in the presence of triphenylethylene (0.7 mg) and then injected into the GLC. A plot of the peak area ratio of II to the internal standard against the concentration of I showed a linear relationship. The linear plot showed a negative intercept indicating column adsorption of II.

Scheme I shows the most probable pathway in the preparation of II. The structure of II was elucidated from IR, NMR, and mass spectrometric analyses. In addition, elemental analysis was performed on the sample.

[2] Applied Science Laboratories.
[3] Fisher Scientific Co.
[4] Eastman Kodak Co.
[5] Hewlett-Packard, 7620A.
[6] Hewlett-Packard, 3370A.
[7] SE-30, Analabs.
[8] Chromosorb W-HP, John-Manville Products Corp.
[9] Perkin-Elmer model 283B.
[10] Varian model T-60.
[11] Finnigan 3300 mass spectrometer.



Scheme I

The IR spectrum of II showed a band at 3300 cm^{-1} representing —NH stretch of a secondary amine, a strong band at 1620 cm^{-1}, and a doublet at 1700 cm^{-1}, indicating carbonyl and —C=N stretches.

The NMR spectrum of II showed six protons of two methyl groups on the aromatic ring at 2.20 ppm (singlet), three protons of the methyl group (NH—CH_3) at 3.10 ppm (doublet), three protons of the aromatic ring at 7.26 ppm (singlet), and one proton (—N=CH—N=) at 7.75 ppm (singlet). The spectrum also reveals additional small signals at 3.15 ppm (doublet) and at 7.95 ppm (singlet). The appearance of the additional signals indicates that II can exhibit tautomerism causing protons of the NH—CH_3 and —N=CH—N= groups to be magnetically nonequivalent and therefore in different environments.

The chemical-ionization mass spectrum of II showed mass peaks at *m/z* 231 (M + 1), 259 (M + 29) and 271 (M + 41) which are characteristic peaks formed with methane as a reagent gas. The spectrum confirmed the molecular weight of the derivative as 230.

Based on the spectral data, the structure of the derivative is 1-(2,6-dimethylphenyl)-4-methylamino-dihydro-1,3,5-triazine-2-one. The elemental analysis of the derivative was in agreement with the chemical composition.

Anal.—Calc. for $C_{12}H_{14}N_4O$: C, 62.59; H, 6.13; N, 24.33. Found: C, 62.54; H, 6.14; N, 24.28.

The derivatization process is shown in Scheme I. The amidinourea moiety of I can be drawn in many tautomeric configurations, one of which is Ia. The tautomer would react with *N,N*-dimethylformamide dimethylacetal to form the *N*-dimethylaminoethylene derivative, an intermediate, which imparts structural stability by ring closure to form II. The cyclization of I to a triazinone using *N,N*-dimethylformamide dimethylacetal reagent presents a new route for the preparation of II (13).

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