Drug Development and Industrial Pharmacy, 22(9&10), 873-880 (1996)

Physicochemical Characterization of L-691,121, a Potent and Selective Class III Antiarrhythmic Agent

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ABSTRACT

The physicochemical properties of the novel antiarrhythmic agent L-691,121 [methanesulfonamide, N-(1'-(2-(5-benzofurazanyl)ethyl)-3,4-dihydro-4-oxospiro(2H-1-benzopyran-2,4'-piperdin)-6-yl), hydrochloride] have been studied, with particular emphasis on the stability, solubility, and dissolution properties of the drug. Solubility data in the pH range 0.5 to 11 show several distinct regions which are consistent with the existence of dicationic, cationic, neutral, and anionic forms of the drug. Dissolution studies were carried out using a continuous flow system which employed a conductance flow cell to monitor dissolved drug concentration. It is shown that the surface area of the drug is an important property in determining the dissolution rate. Solution stability studies indicate that the drug is prone to undergo a base-catalyzed elimination reaction which produces an N-dealkylated product. The preformulation data are used to develop intravenous and oral dosage forms suitable for clinical use.

INTRODUCTION

Class III antiarrhythmic agents are drugs whose predominant mechanism of action involves prolongation of the ventricular refractory period (1). Since cardiac tissue is unresponsive to a second electrical impulse during the refractory period, class III agents are useful for the treatment of ventricular arrhythmias with a reentrant mechanism (2). In particular, sufficiently potent and selective class III agents may find utility in the prevention of sudden cardiac death (SCD) resulting from ventricular tachyarrhythmia. Mortality due to SCD is esti-

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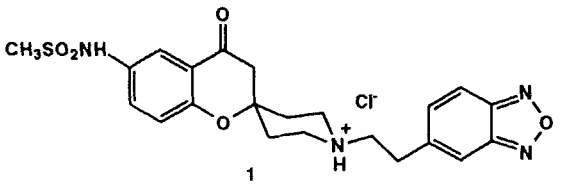
mated at 400,000 deaths annually in the U.S., and a safe and effective drug for this indication would meet a major unsolved medical problem.

L-691,121 (**1**) is a methanesulfonanilide derivative which has been shown to possess intrinsic in vitro and in vivo class III activity (3). Prior to the development of intravenous (IV) and oral dosage formulations, a preformulation study of **1** was initiated in order to elu-



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cidate the key physical and chemical properties of this structurally novel molecule. Initial screening studies suggested that the development of an IV formulation would be complicated by the relatively low and pH-dependent solubility of the drug, and by instability in solution. In contrast, the solid state stability of **1** appeared to be adequate for use in an oral dosage form. However, dissolution rates of the solid drug appeared to be slow and this presented some concern in regard to pharmaceutical processing, as well as the in vivo performance of a solid oral dosage form. Based on these results, an extensive preformulation study was initiated with particular emphasis on the solution and solid-state properties referred to above. The results of these studies, which culminated in the development of oral and IV dosage forms suitable for clinical trials, are described in this report.

MATERIALS AND METHODS

Instrumentation

Drug analyses were performed by reverse-phase high-performance liquid chromatography (HPLC) using a Hewlett Packard 1090 liquid chromatograph equiped with a Spectroflow 757 UV detector (ABI Analytical). Chromatograms were quantitated and stored with a Spectra Physics SP4270 computing integrator. Ultraviolet (UV) spectra were obtained on a Perkin-Elmer Lambda 6 spectrophotometer. An Orion Model 901 Research Meter was used for pH measurement. Drug dissolution experiments were performed in a continuous flow system using a YSI Model 3446 flow conductance cell (cell constant = 0.10 cm^{-1}) connected to a YSI Model 35 conductance meter.

Materials

All solvents were obtained from Fisher Scientific and were HPLC grade or equivalent. Distilled water was taken from a Millipore Milli-Q water purification system. Buffer components were obtained from Fisher Scientific and were Certified ACS grade. L-691,121 was obtained from the Medicinal Chemistry Department of Merck, Sharp, and Dohme Research Laboratories and was greater than 99% pure by HPLC analysis. Thermal analysis and x-ray powder diffraction studies indicated that the compound is an anhydrous crystalline material that exists in only one crystal form.

Solubility Determinations

Equilibrium solubility values were obtained at ambient temperature (22°–23°C) by stirring an excess of the drug suspended in the appropriate buffer solution. At periodic intervals (generally 1–2 days) aliquots were removed and centrifuged, and the supernatant was analyzed by HPLC after dilution. This procedure was continued until two successive measurements gave identical results within experimental error.

Dissociation Constant

The pK_a of **1** was determined by UV spectrophotometry following standard literature procedures (4). The analytical wavelength for the determination was 236 nm.

Solution Stability

The solution stability of **1** was studied in the pH range 1–10 using the following buffer solutions: pH 1.0, hydrochloric acid/sodium chloride; pH 3.0, phosphoric acid/sodium phosphate monobasic; pH 5.0, acetic acid/sodium acetate; pH 7.0, sodium phosphate monobasics/sodium phosphate dibasic; pH 10.0, sodium bicarbonate/sodium carbonate. Except for the pH 1 buffer, the buffer concentration was 0.010 M and sufficient sodium chloride was added to adjust the ionic strength to 0.10 M. Due to solubility limitations, these studies were conducted at very dilute initial drug concentrations (ca. 0.010 mg/ml).

The general procedure for the solution stability studies was the same at all pH values. A stock solution of the drug in the buffer was prepared and several aliquots were immediately analyzed to provide a value for the initial drug concentration. The remainder of the stock

solution was subdivided among glass break-seal ampoules. The ampoules were flame-sealed and stored in the dark in constant temperature (±1°C) ovens at 80°C. Samples were removed at recorded time intervals and the solutions were analyzed for intact drug by HPLC.

Photolytic Stability

Stock solutions were prepared and subdivided as described above, and the solutions were stored approximately 10 in. from an intense (36,000 lumens/ft^2) fluorescent light source enclosed in a reflective cabinet. After 24 hr the samples were removed from the light source and analyzed by HPLC. The temperature within the light cabinet was measured to be 24 ± 1°C.

Decomposition Product Isolation

A sample of **1** (0.025 mg/ml) was dissolved in 500 ml of 0.01 M sodium carbonate buffer at pH 10, and the ionic strength was adjusted to 0.10 M with sodium chloride. This solution was stored in the dark at 80°C for 2 weeks, after which time approximately 60% of the drug had degraded to predominantly one product. The degraded solution was adjusted to pH 7 with concentrated HCl, then extracted with ether to remove residual **1** from the aqueous phase. Isolation of the degradate from the aqueous phase was accomplished using solid-phase extraction on Baker C18 cartridges. The cartridges were first eluted with water to remove buffer components and polar impurities. The degradate was eluted from the column with methanol, the methanol was removed under a stream of dry nitrogen, and the residue was dried in a vacuum oven for 12 hr at 40° to give an off-white solid which was used for structure determination (see later).

Dissolution Studies

The dissolution rate of neat solid **1** was determined by monitoring the conductance of rapidly stirred mixtures of the drug in water using a flow conductance cell. In a typical experiment, 500 ml of distilled water was magnetically stirred at 1100 rpm in an Erlenmeyer flask at room temperature. A peristaltic pump was turned on to initiate flow at 5.0 ml/min from the flask through the conductance cell and back to the flask. The conductance due to pure water was monitored until a stable baseline reading was achieved. A sample (100 mg) of **1** was then quickly added to the flask in a single portion, and the increase in conductance due to the dissolution of the drug was monitored until all the drug was dissolved and the conductance was constant. The output from the conductance meter was sent to a plotter so that, after proper calibration, a continuous plot of solution concentration (or percent dissolved) versus time was obtained from each experiment.

Analytical Methods

Quantitative analysis of **1** was accomplished by isocratic reverse-phase HPLC using a Whatman Partisil 5ODS3 column (25 × 0.46 cm, 5 μm) with a mobile phase of 75% of 0.05 M sodium phosphate monobasic with 0.1% phosphoric acid and 25% acetonitrile at a flow rate of 1.5 ml/min with UV detection at 225 nm. Under these conditions **1** elutes with a retention time of 11–12 min. For quantitation of the decomposition product, the same column and mobile phase composition was used, but the elution pattern was changed to a gradient from 90% to 60% aqueous over 25 min. This gave retention times of 18–19 min for **1** and 6–7 min for the decomposition product.

RESULTS AND DISCUSSION

Solubility Studies

The solubility of **1** in distilled water was determined to be 0.57 mg/ml and the pH of the solution is 5.3. In isotonic saline (0.15 M sodium chloride) a solubility of only 0.020 mg/ml is attained; the solubility depression in saline relative to water is attributable to the common ion effect.

Solubility data for **1** in buffered solutions spanning the pH range of 0.5 to 11.0 are given in Table 1, and a plot of these data is shown in Fig. 1. The graph exhibits several distinct regions which arise as a consequence of the various states of ionization of compound **1**. In the pH region near neutrality, **1** exists either as a cation or as a neutral molecule, and this is reflected in the steep decrease in solubility between pH 5 and 7. Between pH 2 and 5, the compound exists almost completely as a monocation, and the solubility is essentially constant in this pH range. Above pH 7 the free base is the predominant species and the solubility is at a minimum in the pH range of 7 to 8. Interestingly, the solubility of **1** exhibits further variations at pH values less than 2 or greater than 8. This suggests that further ionic equilibria in addition to the monocation/free-base equi-

Table 1

Solubility Data for L-691,121 (1) as a Function of Solution pH

pH	Solubility (mg/ml)
0.50	1.21
1.0	1.07
2.0	0.87
3.0	0.92
4.0	0.93
5.0	0.85
5.5	0.47
6.0	0.20
6.5	0.087
7.0	0.014
7.5	0.016
8.0	0.0080
9.0	0.040
9.8	0.030
11.0	0.21

librium are occurring at the extreme pH values. Overall, the pH-solubility profile is consistent with the equilibria indicated in the accompanying scheme. Note that in this scheme the neutral species 1° is represented as a zwitterion. This is consistent with the acidity constants of the representative model compounds *N*-phenylmethanesulfonamide, pK_a = 8.8 (5) and *N*-methylpiperidine, pK_a = 10.4 (6). The apparent pK_a of **1** was determined to be 7.9 ± 0.1 by UV spectrophotometry.

Due to the anticipated use of **1** in oral therapy, it was of interest to investigate the solubility properties of the compound under conditions of pH and salt concentration which model the gastrointestinal tract. In particular, we wished to reproduce the effect of physiological chloride ion concentration since chloride ion acts as an efficient

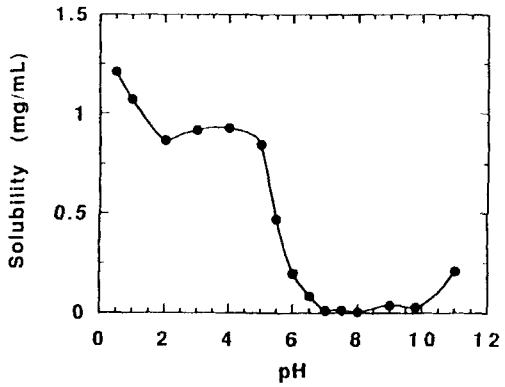


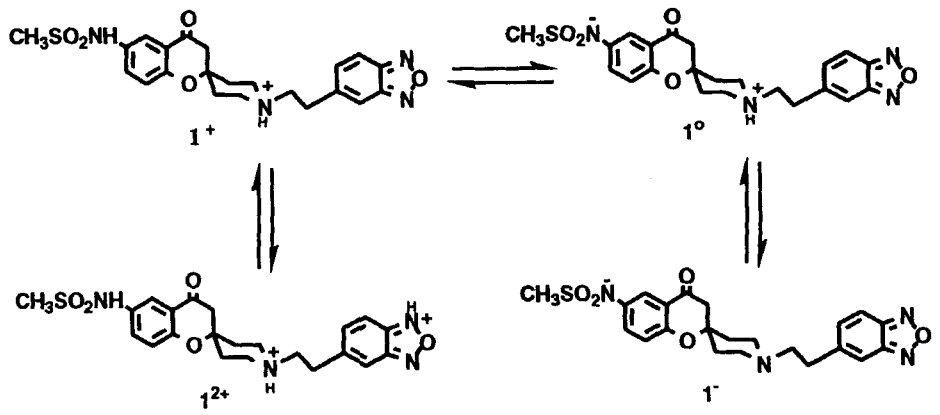
Figure 1. Solubility of **1** as a function of pH in buffered solution.

salting out agent for **1**. Solubility data were obtained in the pH range 2–8 at a constant chloride ion concentration of 0.15 M, which is representative of the chloride level throughout the GI tract (7). These data are shown in Table 2 and indicate that the drug solubility is virtu-

Table 2

Solubility Data for 1 as a Function of pH in 0.15 M Sodium Chloride

pH	Solubility (mg/ml)
1.0	0.026
2.0	0.015
3.0	0.030
4.0	0.017
5.0	0.022
6.0	0.019
7.0	0.014
8.0	0.020



ally independent of pH in the presence of chloride ion. This reflects the facts that at low pH the compound exists as a hydrochloride salt and is subject to salting-out by the common ion effect, and that at high pH the compound exists in the intrinsically insoluble neutral (zwitterionic) form.

Dissolution Studies

In view of the generally low solubility of **1**, it was deemed useful to characterize the dissolution rate of the drug powder. To accomplish this, we have used a continuous flow method in which the concentration of dissolved drug is monitored by the electrical conductance of the solution using a flow conductance cell. Since undissolved drug particles do not effect the solution conductance, filtration of discrete samples is unnecessary and a continuous trace of dissolved drug versus time is obtained. In this respect, the flow conductance method has a significant advantage over the usual dissolution procedure involving sampling, filtration, sample preparation, and assay by UV or HPLC. Of course, the conductance method can only be used with compounds that are capable of carrying an electrical charge.

A typical output from a dissolution experiment is shown in Fig. 2. Despite the low solubility of **1**, dissolution was generally observed to be relatively rapid, with 50% of the drug dissolving in 1 min and 90% in 8 min (Fig. 2, A). However, certain drug lots were observed to dissolve at significantly slower rates (Fig. 2, B). Characterization of these samples by x-ray methods indicated that all the lots used in the dissolution studies

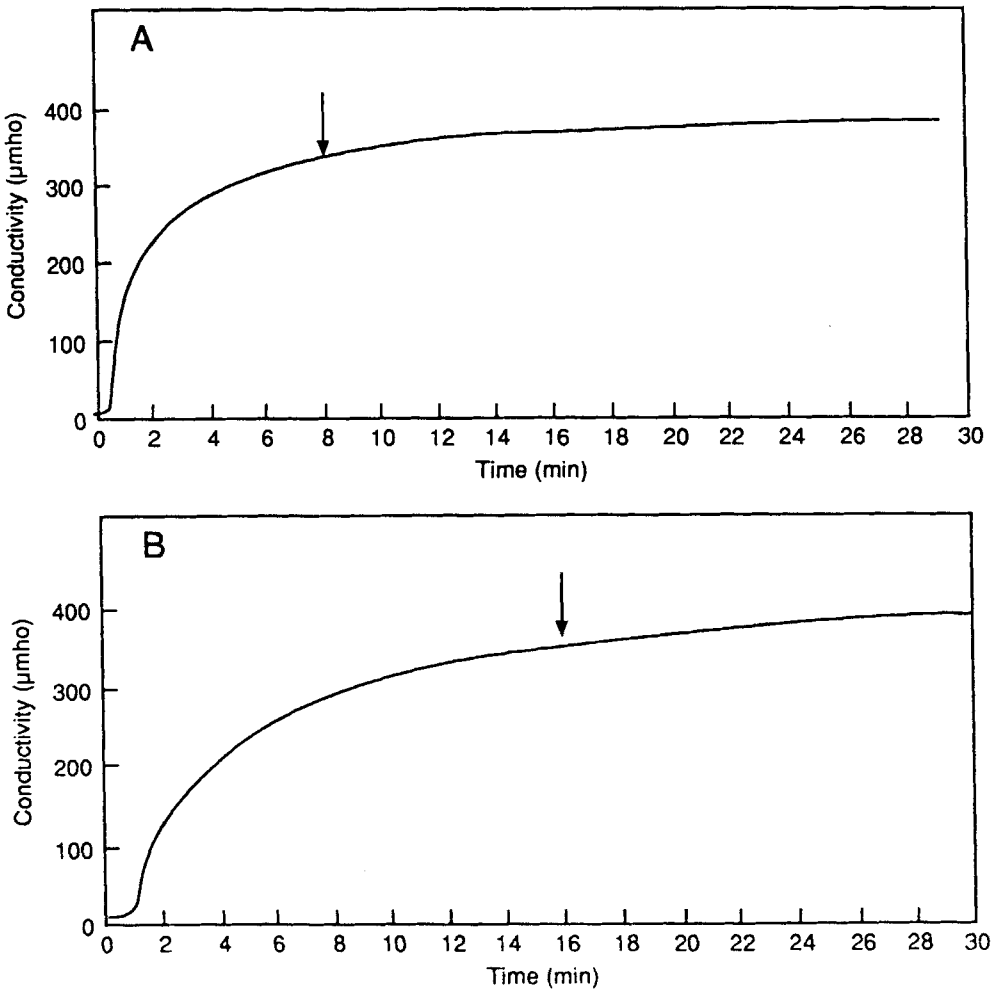


Figure 2. Dissolution profiles of **1** plotted as solution conductance (μmho) vs. time for (A) "fast"-dissolving sample, and (B) "slow"-dissolving sample. The arrows denote points at which 90% of the sample is dissolved.

exhibited identical diffraction patterns. Among the physical properties examined, only the surface area of the drug correlated with the results of the dissolution studies. Data on the surface area and dissolution rate of four distinct samples obtained from various processes are shown in Table 3. Based on these data, it was recommended that drug samples adhere to a surface area requirement of >2 m^2/g in order to assure adequate dissolution.

Table 3

Dissolution Rate (Time to 90% Dissolution, $t_{90\%}$) and Surface Area Data for Samples of 1

Sample	$t_{90\%}$ (min)	Surface Area (m^2/g)
A	8	2.35
B	8	2.39
C	16	1.52
D	70	0.403

Stability Studies

The solution stability of **1** was investigated at 80°C in the pH range 1–10, and the data are presented in Fig. 3 and Table 4. Note that the drug exhibits remarkable stability in strongly acidic solution, with little or no decomposition occuring at pH 1 and 3 over a period of 7 weeks. However, the data reveal that drug decomposition does occur in solutions at pH of 5 or greater, and that the rate of drug loss is consistent with first-order kinetics. Moreover, chromatographic analysis of degraded samples indicates that **1** decomposes to give predominantly a single product. Isolation of this degradate was accomplished by liquid extraction with ether (to remove unreacted **1** in the ether phase) followed by solid-phase extraction to remove buffer components and leave a relatively pure single component (Fig. 4). A structural assignment was made on the basis of the low resolution electron impact mass spectrum, which shows a molecular ion peak at *m/e* 310 and a base peak at *m/e* 231. This suggests that **1** undergoes a base-catalyzed elimination reaction to yield the *N*-dealkylated product **2** as shown. The mass spectral data for **2** are consistent with the accompanying fragmentation pattern.

Final confirmation of structure was made by comparison of UV and infrared (IR) spectra with an authentic sample of **2**, which is an intermediate in the synthesis of **1**. In particular, it was demonstrated that the isolated

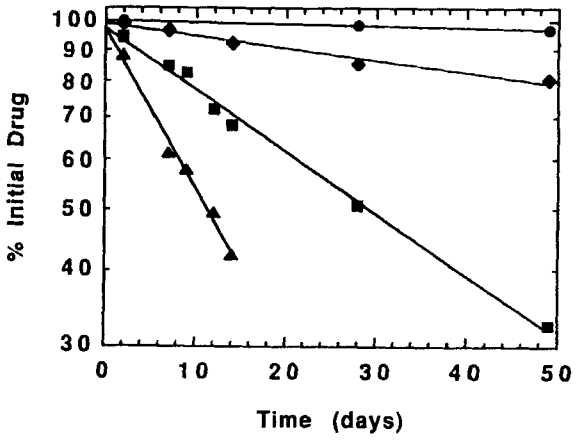


Figure 3. Solution stability data for **1** at 80°C, expressed as % of initial drug remaining vs. time. Circles, pH 3.0; diamonds, pH 5.0; squares, pH 7.0; triangles, pH 10.0.

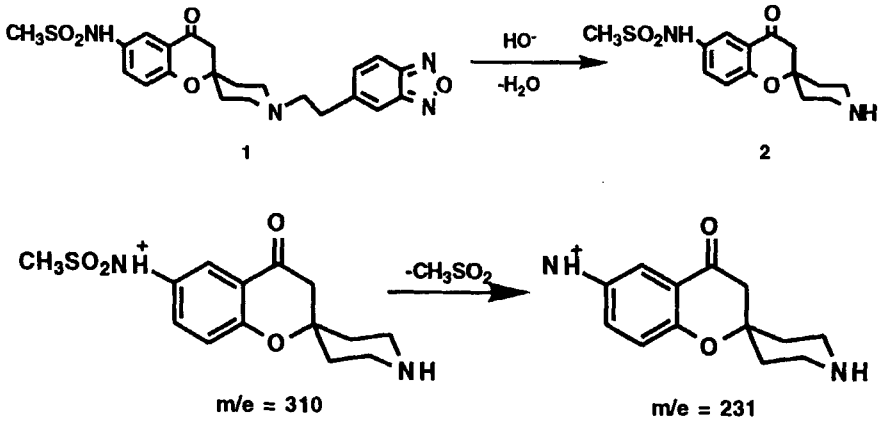


Table 4

Observed Rate Constants and Half-Lives for 1 in Buffered Solutions at 80°C

pH	$k \times 10^2$ (Days^{-1})	$t_{1/2}$ (Days)
1.0	[a]	—
3.0	0.0634[b]	1093
5.0	0.583	119
7.0	2.28	30.4
10.0	5.87	11.8

[a]No decomposition was observed at this pH after 49 days.
[b]Approximate value based on initial rate of reaction.

degradate and authentic **2** have identical retention times on gradient elution HPLC as shown in Fig. 4. The formation of **2** is expected to be accompanied by the formation of vinylbenzfurazan **3** in an equimolar amount. Surprisingly, we have been unable to chromatographically detect the presence of **3** in degraded solutions of **1**; it is speculated that the vinylbenzfurazan, due to its styrene-like structure, may undergo polymerization under the reaction conditions.

In addition to undergoing base-catalyzed thermal decomposition, compound **1** was observed to be extremely photolabile in solution. Table 5 summarizes several experiments which indicate that the photodecomposition is unaffected by the atmosphere (air vs. nitrogen), and that the rate of photodecomposition is concentration dependent, with more dilute drug solutions being less stable. Of practical importance, it was demonstrated that the simple expedient of storing the drug solutions in amber vials was fully effective in preventing degradation. Chromatograms of photodegraded samples revealed the presence of multiple decomposition products with no single product accounting for more than ca. 10–15% of the overall distribution. Photodecomposition of **1** presumably involves the benzfurazan portion of the molecule (8), but the complicated product mixture suggests that numerous secondary and/or parallel processes are occurring.

In the solid state, compound **1** was found to be extremely stable as a neat solid and in combination with pharmaceutical excipients. No drug decomposition was observed from samples of the neat solid drug from dry powder mixtures with lactose, microcrystalline cellulose, pregelatinized starch, and magnesium stearate after storage for up to 12 weeks at 80°C.

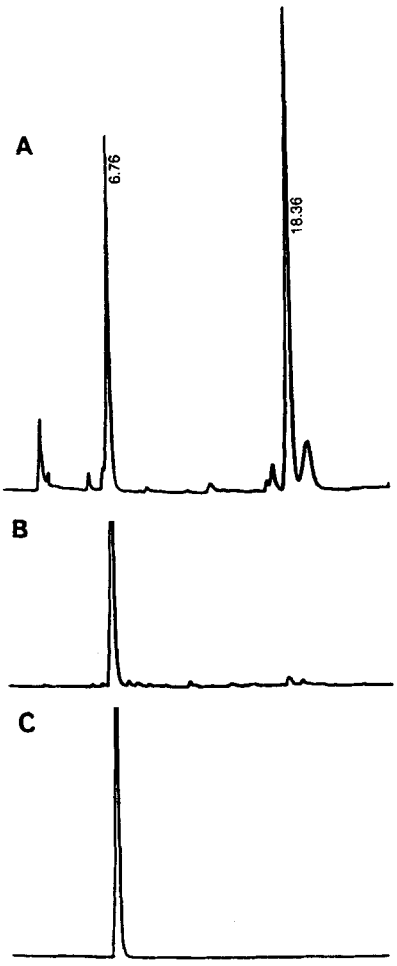


Figure 4. HPLC chromatograms of (A) degraded sample of **1**, (B) isolated degradate, and (C) authentic sample of degradate.

Table 5

Stability of 1 After Exposure to Fluorescent Light (36,000 lumen/ft^2) for 24 hr[a]

C_0 (mg/ml)	Container[b]	Atmosphere	% Initial Drug
0.50	Clear	Air	95.4
0.50	Clear	Nitrogen	95.5
0.025	Clear	Air	79.1
0.50	Amber	Air	101

[a]Solutions in a pH 5.0 citrate buffer were stored approximately 10 in. from the light source.
[b]Wheaton type I borosilicate glass container, clear or amber-tinted glass as indicated.

CONCLUSIONS

The preformulation data for **1** were used to develop both IV and oral dosage forms for clinical use. For the IV formulation, the principal concerns uncovered in our studies relate to solubility and solution stability. The data in Tables 1 and 4 indicate that the optimal formulation pH would be about 3; this, however, was considered too acidic for IV administration and an IV formulation buffered to pH 5 was considered to be the best compromise between stability, solubility, and tolerability factors. Due to the salting-out effect, the formulation uses mannitol rather than sodium chloride for tonicity adjustment. In accordance with the photolytic stability results, the IV solutions are packaged in amber vials.

Stability studies with solid-excipient mixtures did not reveal any incompatibilities which would be a concern in oral dosage form development. However, the powder dissolution results did indicate that slow and variable dissolution of the drug from a solid dosage form was a potential concern. As suggested by the data in Table 3, only samples of **1** with an appropriate particle size were used for the manufacture of a capsule formulation. The capsule and IV solution formulations are currently being used to assess the clinical utility of **1**.

ACKNOWLEDGMENTS

The authors wish to thank Drs. Dave Claremon, Jason Elliot, and John Butcher for providing an authentic sample of compound **2**, and for their helpful discussions. We also thank Dr. Harry Ramjit for providing the mass spectral data, and Mr. Raymond Higgins for providing the surface area measurements.

REFERENCES

1. J. R. Harper and L. S. Gettes, Cardiovasc. Rev. Rep., 9, 59 (1988).
2. H. J. J. Wellens, P. Brugada, and J. Farre, Am. Heart J., 107, 1053 (1984).
3. J. M. Elliot, J. J. Baldwin, S. A. Buhrow, D. A. Claremon, J. J. Lynch, G. S. Ponticello, E. M. Radzilowski, D. C. Remy, H. G. Selnick, R. Stein, J. I. White, and M. B. Young, Development of L-691,121, Book of Abstracts, 192nd ACS National Meeting, New York, 1991.
4. A. Albert and E. P. Serjeant, *The Determination of Ionization Constants*, Chapman and Hall, London, 1984, p. 70.
5. R. D. Trepka, J. K. Harrington, and J. W. Belisle, J. Org. Chem., 39, 1094 (1974).
6. D. D. Perrin, *Dissociation Constants for Organic Bases in Aqueous Solution*, Butterworths, London, 1965, p. 137.
7. J. G. Banwell, S. L. Gorbach, N. F. Pierce, R. Mitra, and A. Mendal, J. Clin. Invest. 50, 890 (1971).
8. I. Yavari, S. Esfandiari, A. J. Mostashari, and P. W. W. Hunter, J. Org. Chem., 40, 2880 (1975).