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Identification and Analysis of Persistent Contaminants Associated with Gemcadiol

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Abstract □ Application of several analytical probes indicated that certain batches of the investigational new drug gemcadiol (2,2,9,9-tetramethyl-1,10-decanediol) were contaminated with impurities whose nature and source were unknown. Subsequent study showed that these impurities consisted of polymeric material formed by self-condensation of the dialdehyde precursor. Gel permeation chromatographic techniques were found to be useful in the analysis of polymers present in both the aldehyde precursor and the finished drug.

Keyphrases □ Gemcadiol—identification and analysis of persistent contaminants □ Drug impurities—gemcadiol, identification and analysis of persistent contaminants □ Cholesterol-lowering agents, potential—gemcadiol, identification and analysis of persistent contaminants

Gemcadiol (2,2,9,9-tetramethyl-1,10-decanediol, I) is an investigational new drug that is being evaluated as a triglyceride- and cholesterol-lowering agent (1). This drug may be synthesized by a process whose last step involves sodium borohydride reduction of 2,2,9,9-tetramethyl-1,10-decanedial (II) (2).

When various batches of I were analyzed, it became apparent that significant interbatch variation existed for which the cause was not known. This study was conducted to determine the reason for the observed inconsistencies and to provide a more definitive method of analysis.

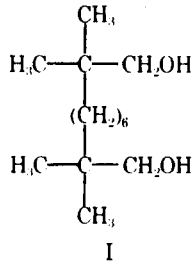
EXPERIMENTAL

Reagents—The solvents were analytical reagent grade. The sodium borohydride[1] was used as received.

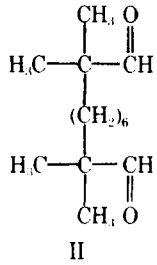
Apparatus—IR spectra were obtained using a grating IR spectrophotometer[2]. GLC was done on a programmable research chromatograph[3] equipped with a flame-ionization detector and an electronic integrator[4]. NMR spectra were obtained on a 90-MHz instrument[5] with deuterochloroform as the solvent and tetramethylsilane as the internal standard.

GLC Studies—GLC studies were performed using 183 × 0.64-cm stainless steel columns packed with 5% silicone gum rubber[6] on silanized diatomaceous earth[7]. Chromatograms were obtained under isothermal conditions (225°) after injection of 2 μl of a 10% solution of the sample in methanol. In certain cases, the solution injected was acidified by adding 1 drop of 3 *N* HCl to 1 ml of the methanolic sample solution.

Liquid Chromatography of I—Liquid chromatographic separation of the components present in impure samples of I was accomplished using modified dextran beads[8] packed in a 40 × 3-cm glass chromatographic column. Methanol (25% v/v) in benzene was the mobile phase. The column was charged with a sample of ~500 mg dissolved in 5 ml of the mobile



I



II

[1] Alfa Inorganics, Ventron Corp.

[2] Model 457, Perkin-Elmer Corp.
[3] Model 5750, F & M Scientific Corp.
[4] Model 3370A, Hewlett-Packard Corp.
[5] Bruker WH 90.
[6] OV-17, Hewlett-Packard.
[7] Chromasorb G, Hewlett-Packard.
[8] Sephadex LH-20, Pharmacia Fine Chemicals.

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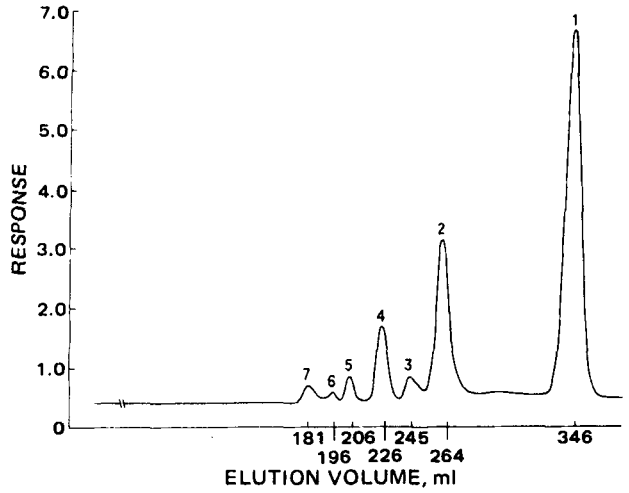


Figure 1—*Liquid chromatogram of polymerized II obtained on a column packed with Bio Beads SX-2 using benzene as the eluent. Elution volumes of each peak are indicated. The indicated molecular weights are: peak 2, 610–690; peak 3, 750–840; peak 4, 940–1060; peak 5, 1200–1400; and peak 6, 1300–1600.*

phase; it was operated at a flow rate of 1 ml/min, and 10-ml fractions were collected. The fractions were transferred to tared aluminum weighing pans[9] (70 mm), the eluent was allowed to evaporate, and the pans were brought to constant weight under a vacuum. The weight of nonvolatile material in each pan then was plotted *versus* the eluent volume to obtain the chromatogram.

Liquid Chromatography of II—The chromatographic system employed for II was essentially the same as that developed by Mulder and Buytenhuys (3). Glass columns (90 × 2.4 cm) packed with porous styrenedivinylbenzene beads[10] were employed with benzene as the mobile phase. The columns were attached to a commercial high-efficiency liquid chromatographic system[11] equipped with a refractive index detector[12]. Five milliliters of a 5% sample solution in benzene was introduced to the column with a sample injection valve[13]. The columns were operated at a flow rate of 20 ml/hr at ambient temperature.

Estimation of Molecular Weights of Components Present in Partially Polymerized II—A mixture containing known compounds of relatively high molecular weights was prepared by dissolving 100 mg of II and 50 mg each of tristearin[14] (mol. wt. 891), trimyristin[14] (mol. wt. 723), and trilaurin[14] (mol. wt. 639) in 5 ml of benzene. A chromatogram was obtained for this mixture using the chromatographic system and conditions described for the liquid chromatography of II. The elution volumes of the known components were noted, and a plot of log mol. wt. *versus* elution volume was constructed.

Although the plot was linear, its slope caused the anticipated molecular weights to be underestimated (specific adsorption effects). Nevertheless, these estimates are the basis for the lower molecular weights of the molecular weight estimate given in Fig. 1. By assuming that the lowest polymeric contaminant of the aldehyde mixture, Component 2, had a molecular weight of 678 and by calibrating the column with this compound and II (mol. wt. 226), the estimates for the upper limit of the range estimate were obtained.

Reduction of Samples of II—In general, 1 g of II was dissolved in 5 ml of 2-propanol and placed in a small beaker containing a magnetic stirring bar. A solution of sodium borohydride (176 mg/g of II) in distilled water was added dropwise with stirring. The resulting mixture was stirred for an additional 30 min.

After the reaction was complete, the mixture was poured into excess water and ice, and this mixture was stirred. The final mixture was extracted with three equal volumes of chloroform, and the chloroform extract was dried over anhydrous magnesium sulfate. After the magnesium sulfate was separated by filtration, the chloroform was evaporated and and the residue was brought to constant weight under vacuum.

Reduction of Components in Impure Samples of II—Each component found in polymerized samples of II was reduced immediately following elution from the chromatographic column by piping the column eluate into separate receivers, each containing 100 mg of sodium borohydride dissolved in water–methanol (10:40). The methanol was evaporated in a rotary evaporator, and excess water was added.

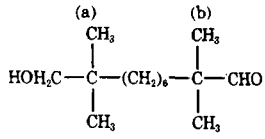
The aqueous mixture was extracted three times with 25-ml portions of chloroform, and the chloroform extracts were pooled. The combined chloroform extracts were back-washed with water until they were neutral to litmus paper and then they were dried over anhydrous magnesium sulfate. After freeing the extract of solid magnesium sulfate, the chloroform was evaporated and the residue was collected.

Preparation and Characterization of 10-Hydroxy-2,2,9,9-tetramethyldecanal (III)—Compound II, 100 g, was dissolved in 128 ml of 2-propanol and was kept stirring in a beaker. A solution of 8.3 g of sodium borohydride in 109 ml of water was added dropwise. At the end of this addition, the reaction mixture was analyzed by GLC and found to be a mixture of 80% I and 20% III. The solvent was evaporated, and the residue was dissolved in warm acetonitrile. Upon cooling, some I crystallized, leaving a supernate containing 32% I and 68% III. Compound III was isolated from the mother liquor as follows.

The solvent was evaporated, and 50 g of the residue was refluxed for 2 hr with 50 g of Girard's Reagent T[15], 50 ml of acetic acid, and 500 ml of 95% ethanol. The alcohol then was evaporated, and the residue was washed three times with 200-ml portions of ether. It then was dissolved in 300 ml of water, and 60 ml of 5 *N* HCl was added. The mixture was heated on the steam bath for 1 hr. The formed oil was separated and dissolved in ether. The ether solution was washed with a dilute potassium bicarbonate solution and then with water. The ether solution was dried over anhydrous magnesium sulfate, and the ether was evaporated.

The residue was free of I and was identified as 10-hydroxy-2,2,9,9-tetramethyldecanal (III). The mass spectrum exhibited a molecular ion

Table I—NMR (Deuterochloroform) Data for III



Functional Group	Chemical Shift, ppm	Relative Area
CH_3 (a)	0.82	6
CH_3 (b)	1.0	6
CH_2 (alkyl)	1.21	12
CH	1.74	1
CH_2 (CH_2OH)	3.27	2
CHO	9.40	1

Table II—Results from Analysis of Various Batches of I by Differential Scanning Calorimetry (DSC) and GLC

Batch	Percent I	
	DSC	GLC
A	99.6	98.0
B	97.4	92.4
C	98.9	97.8
D	—[a]	86.9

[a] Thermogram was excessively distorted so calculation was not possible.

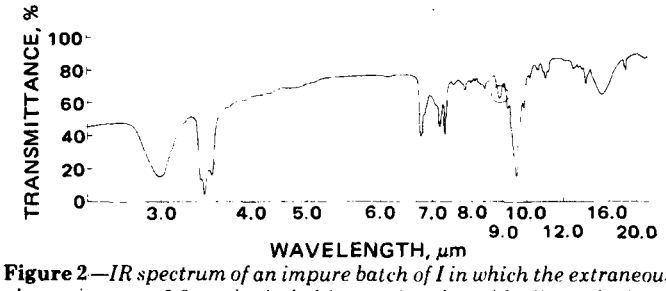


Figure 2—*IR spectrum of an impure batch of I in which the extraneous absorption near 9.0 μm is circled (potassium bromide dispersion).*

[9] Arthur H. Thomas Co.
[10] Bio Beads SX-2, Bio Rad Laboratories.
[11] Series 4100, Varian Aerograph.
[12] Varian Aerograph.
[13] SV-8031, Chromatronix.
[14] Nu Chek Prep, Elysian, Minn.
[15] Aldrich Chemical Co., Milwaukee, Wis.

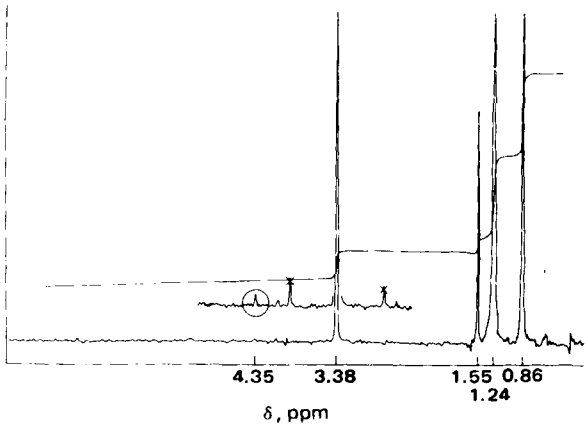


Figure 3—*NMR spectrum of impure I (1% in deuterochloroform) in which the extraneous absorption at 4.35 ppm is circled and where × denotes side bands.*

at *m/e* 228. IR analysis gave a strong band at 5.78 μm due to C=O. GLC gave only one peak. The NMR data are given in Table I.

Anal.—Calc. for $C_{14}H_{28}O$: C, 73.68; H, 12.28. Found: C, 73.60; H, 12.20.

Determination of Amount of Polymeric Contaminants in I—Approximately 10 g of Sephadex LH-20 was conditioned by stirring with benzene–methanol (75:25) for several hours. The solvated beads were packed into a 45 × 2-cm glass column. The height of the resin bed was 42 cm. Approximately 200 mg of the sample was weighed accurately and dissolved in 5 ml of the eluent (benzene–methanol). The solution then was introduced into the column, and the column was eluted with benzene–methanol (75:25).

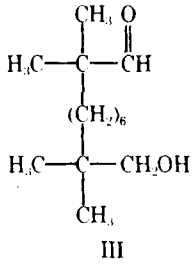
Fractions of 30, 35, and 40 ml were collected and transferred into three tared aluminum pans. The solvent was evaporated, and the pans were dried under vacuum to constant weight. Fractions 2 and 3 contained the total polymeric impurities and the drug, respectively. These fractions were weighed, and the amount of polymeric contaminants in I was calculated from their weights.

RESULTS AND DISCUSSION

Purity estimates of various batches of I, obtained by differential calorimetry[16] (4), are shown in Table II; significant interbatch variation was observed. IR spectra obtained from these batches differed in that the spectrum of the less pure material had extraneous absorption near 9 μm (Fig. 2). The NMR spectra of high and low quality drug were similar (Fig. 3), except for a barely perceptible peak near 4.35 ppm.

Gas–liquid chromatograms obtained from impure batches (Fig. 4, left) were characterized by a large symmetrical peak preceded by a small distorted peak or shoulder. The relative area of the distorted peak or shoulder varied from batch to batch, and its shape suggested it could have arisen from partial thermal decomposition of I itself or associated contaminants. When the methanolic sample solution used for injection was acidified prior to injection, relatively impure samples gave a chromatogram like that shown in Fig. 4 (right). The retention time of two of the observed small peaks corresponded to II and III. The presence of these compounds was confirmed through mass spectrometry and the coincidence of the retention time with authentic samples.

Since the chromatographic behavior of the vapor phase indicated thermal decomposition, other means of separation were investigated. It was determined that extraneous material could be separated from the



III

[16] Model DSC 1B differential scanning calorimeter, Perkin-Elmer.

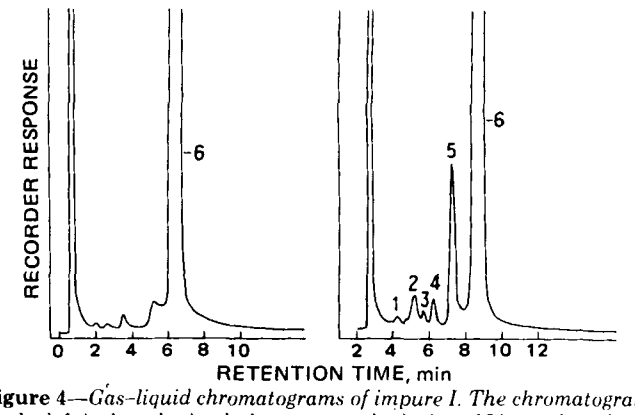


Figure 4—*Gas–liquid chromatograms of impure I. The chromatogram on the left is that obtained when a neutral solution of I in methanol was injected. The chromatogram on the right was obtained when an acidic solution of I in methanol was injected. Peaks 5, 4, and 6 represent III, II, and I, respectively.*

drug through gel permeation chromatography using Sephadex LH-20 with methanol–benzene as the eluent[17]. A typical chromatogram obtained on an impure batch of drug is shown in Fig. 5. IR spectra obtained on the material responsible for the various peaks showed strong absorption at ~9 μm for peaks 1–3, whereas the spectrum of peak 4 had no extraneous absorption and was consistent with the pure drug.

More of the major contaminant (peak 3) was obtained[18] for characterization, and the NMR spectrum was informative (Fig. 6). While the spectrum of the contaminant was similar to that of the drug, the details differed. A significant difference was that the signal from the methyl protons occurred at more than one chemical shift, and there was a small but real peak near 4.4 ppm. The complete NMR data obtained on this sample are summarized in Table III.

Structures A and B are consistent with the NMR data and are chemically reasonable (5–8).

Anal.—Calc. for Structure A or B: C, 73.68; H, 12.28. Found: C, 73.70; H, 12.19.

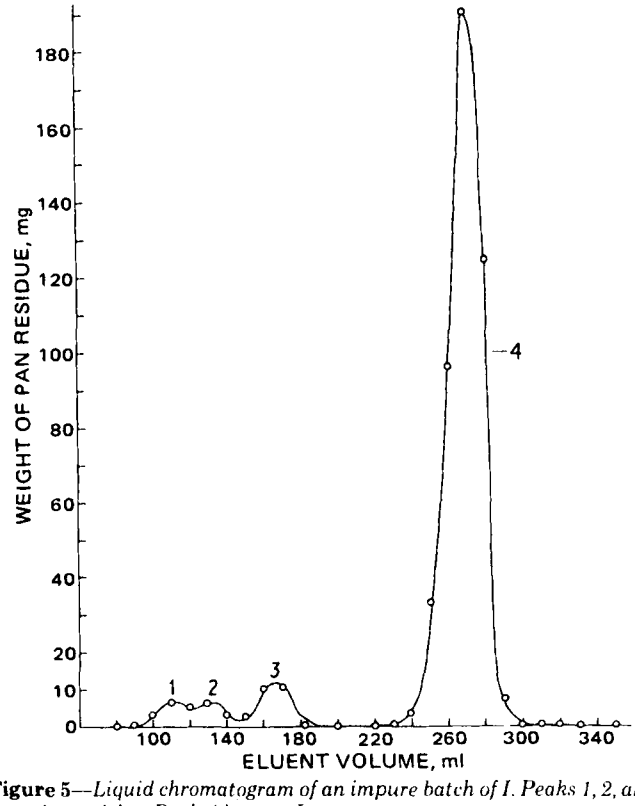


Figure 5—*Liquid chromatogram of an impure batch of I. Peaks 1, 2, and 3 are impurities. Peak 4 is pure I.*

[17] See *Experimental.*

[18] Preparative chromatography using a 45 × 6-cm glass column.

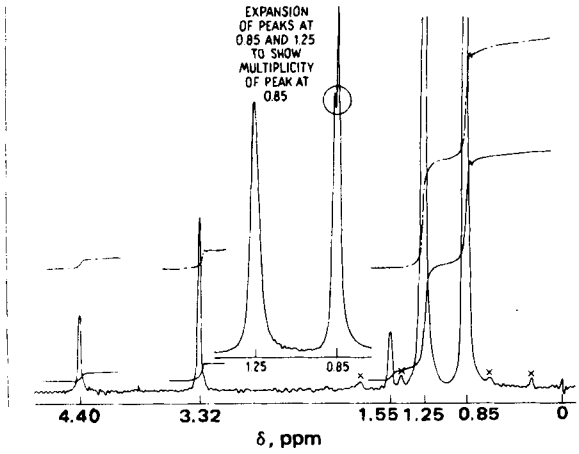


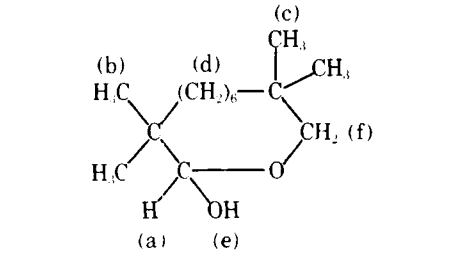
Figure 6—*NMR spectrum of material corresponding to peak 3 in Fig. 5 (2% in deuterochloroform).*

The IR spectrum showed a strong band at 9 μm, suggesting the presence of C—O—C. GLC of an acidified methanolic solution gave only one peak corresponding to 10-hydroxy-2,2,9,9-tetramethyldecanal (III). The molecular weight determined by the Rast procedure (9) was 611 ± 10%. This result and its behavior on the gel permeation column[19] indicate that its molecular weight cannot be 228, so Structure B is favored.

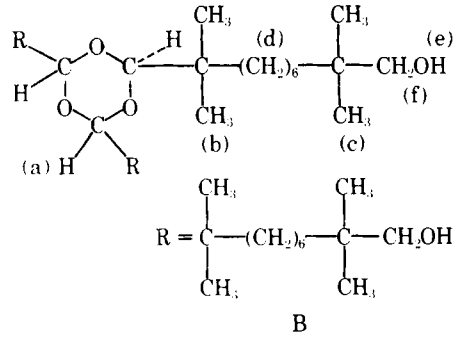
Examination of the IR spectrum of various batches of the dialdehyde precursor (II) revealed that relatively strong absorption near 9 μm existed in some batches but not in others. A sample of dialdehyde giving an IR spectrum that had strong absorption near 9 μm and a sample not having this extraneous absorption were reduced, and the reduction products were chromatographed. The chromatograms obtained showed that impurities were formed only in the case where the dialdehyde gave an IR spectrum with significant absorption near 9 μm.

Since further information regarding the nature of the contaminants in the dialdehyde was desirable, a liquid chromatographic system suitable for their separation was developed and chromatograms were obtained. A chromatogram representative of the dialdehyde with strong 9-μm absorption is shown in Fig. 1. The system employed provided near baseline separation of at least six contaminants.

A range estimate of molecular weights of the various components obtained on the basis of elution volume are included in Fig. 1. Attempts were made to obtain definitive NMR data directly on these contaminants; although peaks were present that could be assigned to ring and aldehyde protons (Table IV), spurious peaks also were observed. These peaks,



A



B

[19] The elution volume of this material is much less than that of II, whose molecular weight is 228.

Table III—NMR Data Obtained from Material Corresponding to Peak 3 in Fig. 5

Functional Group	Chemical Shift, ppm	Relative Area
CH (a)	4.4	1
CH_3 (b,c)	0.85	12.6
CH_2 (alkyl) (d)	1.25	12.6
OH (e)	1.57	1
CH_2 (CH_2OH) (f)	3.3	2

Table IV—NMR Data Obtained from Material Corresponding to Peak 4 in Fig. 1

Functional Group	Chemical Shift, ppm	Relative Area
CH	4.4	7.5
CH_3	(0.90) −(1.05)	72
CH_2	1.21 1.23 1.29 1.49	122
?	8.04	2
CHO	9.48	3

Table V—NMR Data Obtained from Material Corresponding to Peak 4 in Fig. 1 after Reduction with Sodium Borohydride

Functional Group	Chemical Shift, ppm	Proton Ratio
CH	4.37	1.44
CH_3	0.86	14.2
CH_2 (alkyl)	1.23	14.6
OH	1.67	1.0
CH_2 (CH_2OH)	3.29	1.85

which resulted from oxidation, precluded clear definition of proton ratios.

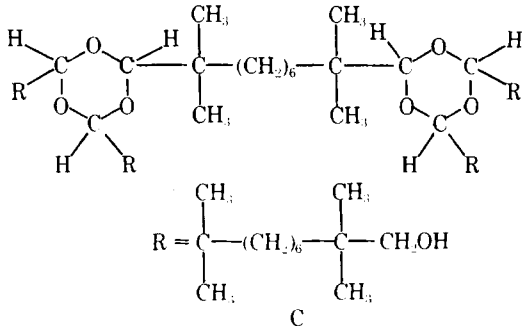
Since the components of the impure dialdehyde were well separated, they were reduced concurrently with elution, and an impure drug sample was spiked with the reduced aldehyde impurities. Liquid chromatograms then were obtained, which indicated that the material responsible for peak 2 (Fig. 1) corresponded to drug contaminant 3 (Fig. 5) and that aldehyde contaminant 4 (Fig. 1) corresponded to drug contaminant 2 (Fig. 5). Since components of the impure dialdehyde were better separated than their reduced counterparts, a sufficient sample of a minor drug contaminant, peak 2 (Fig. 5), was obtained by chromatographing partially polymerized dialdehyde and providing concurrent reduction. This material was a viscous clear liquid.

Anal.—Calc. for $C_{70}H_{138}O_{10}$ (Structure C): C, 73.81; H, 12.13. Found: C, 73.50; H, 12.09.

The molecular weight calculated for Structure C was 1138; the range indicated by gel permeation chromatography was 940–1060.

GLC of the acidic methanolic solution gave both the monoaldehyde monoalcohol (III) and the dialdehyde in a molar ratio of 4:1. The presence of the dialdehyde is significant and indicates that this higher molecular weight impurity contains a dialdehyde bridge incorporated so as to protect both potential aldehyde groups from reduction. The NMR data are presented in Table V.

These properties are consistent with and indicative of Structure C as the structure of the next most abundant drug contaminant.



C

Table VI—Theoretical Proton Ratios for Various Protons Involved in Polymeric Alcohols Containing Various Numbers of Trioxane Rings

Protons	Proton Ratios (Group per OH)			
	1 Ring	2 Rings	3 Rings	4 Rings
Ring CH	1.0	1.5	1.8	2.0
Aliphatic CH_2	12.0	15.0	16.8	18.0
Methyl	12.0	15.0	16.8	18.0

The ratio of various protons having different chemical shifts would be expected to vary as polymerization continued. In particular, the ratio of ring CH, aliphatic CH_2, and methyl protons to hydroxyl protons would increase as the number of bridged trioxane rings increased (Table VI).

While it was important to separate individual components for characterization, this need does not apply for the establishment of drug purity. The gel permeation technique was modified to allow analysis of the total polymeric material in drug samples. To validate this method of analysis, mixtures having known compositions were analyzed. The results (percent impurity added: 0, 5.2, and 9.4; found: 0, 5.1, and 9.4) indicate that the method described under *Experimental* is sufficiently accurate for use.

CONCLUSIONS

Available information indicates that contaminants present in certain batches of I consist of polymeric alcohols characterized by at least one trioxane ring. These contaminants originate in the dialdehyde precursor, which may polymerize to form corresponding compounds. Reduction of polymers present in the dialdehyde yields the alcohol polymers, which may persist throughout purification and contaminate the finished drug. Analysis *via* gel permeation liquid chromatography provides a useful means to analyze the degree of contamination by these polymeric compounds.

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Preliminary Model for Streptozocin Metabolism in Mice

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Received June 25, 1979, from the **Department of Chemical Engineering, Columbia University, New York, NY 10027*, and the ‡*Department of Chemical Engineering, Carnegie-Mellon University, Pittsburgh, PA 15213.* Accepted for publication January 18, 1980.

Abstract □ A mathematical model for streptozocin metabolism in mice is presented. By using the available bioassay and chemical assay data for a 300-mg/kg ip injection and the principles of physiologically based pharmacokinetics, a membrane-limited transport model with first-order kinetics was found to simulate the data adequately (average error of <20%). Furthermore, the first-order reaction constant derived in analyzing the bioassay data (0.009 min^{-1}) was in close agreement with the half-life of streptozocin (1 hr) reported previously.

Keyphrases □ Streptozocin—drug metabolism and distribution, mathematical model based on bioassay and chemical assay, mice □ Metabolism—streptozocin distribution and transport, mice, mathematical model □ Diabetes—streptozocin induced, pharmacokinetic model, mice □ Pharmacokinetic models—streptozocin metabolism, bioassay and chemical assay, mice, membrane-limited transport

Streptozocin (NSC-85998), the 2-deoxy-D-glucose derivative of 1-methylnitrosourea (1), has been shown to inhibit primary DNA synthesis in mammalian cells (2, 3). Due to its selective toxicity for pancreatic β-cells, streptozocin has been used to induce diabetes in a variety of animals (4–6). In addition, it has been useful in the treatment of metastatic insulinoma (7, 8).

BACKGROUND

Previous kinetic studies showed that streptozocin degradation obeys first-order reaction kinetics. These studies also indicated the presence of at least two pathways for streptozocin degradation, one involving the *N*-nitroso group and the other involving another portion of the molecule, probably the glucose moiety (9). While chromatography has been used to isolate several streptozocin metabolites (10, 11), their molecular structures have not been identified precisely.

In addition, data for streptozocin concentrations in various tissues of mice as a function of time were obtained previously (10) using three assay methods: bioassay and chemical and radiochemical assays. The bioassay is the closest indicator of the actual streptozocin content, but this method does not preclude the possibility that some drug metabolites also may be bioactive. The chemical assay measures the *N*-nitroso group of streptozocin and its metabolites. The radiochemical assay measures a tritiated hydrogen atom that originally was at the C-6 position of the glucose moiety of streptozocin and must be accounted for either as part of the streptozocin molecule or as part of the several streptozocin metabolites known to exist.

The objective of this study was to utilize these data along with the present knowledge of streptozocin metabolism to develop mathematical models for the distribution, transport, and metabolism of streptozocin in mice.

THEORETICAL

In recent years, physiologically based pharmacokinetic modeling (12, 13) has been applied extensively to predict drug distributions in plasma and various tissues of mammalian systems (14, 15). A pharmacokinetic model is a simplified representation of a real physical system (Scheme I) derived from experimental observations, previous knowledge, and a number of assumptions. It is based on relevant physical and biochemical parameters such as plasma flow rates, tissue volumes, tissue binding constants, mass transfer coefficients, and, when necessary, chemical reaction constants to account for drug metabolism. The validity of the model as a means of predicting tissue drug distributions and excretion rates is tested by comparing model predictions with data. Plasma flow rates and tissue volumes for most species and tissues are readily available in the literature. Mass transfer, binding, and metabolism parameters for a given drug must be estimated from the available data.

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