THE CHARACTERIZATION AND METABOLISM OF 2,3-DIMETHYL-5,6-DIHYDROPYRAZINE

By

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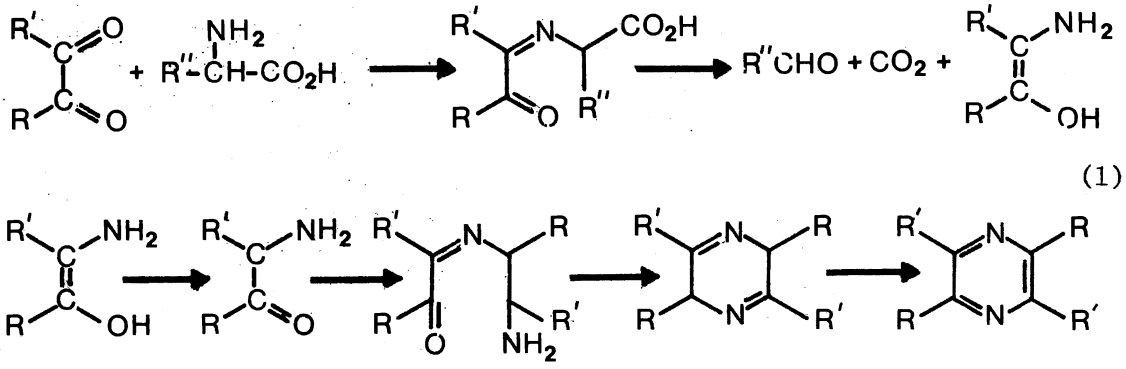
CHAPTER I

INTRODUCTION

Recent advances in the study of food chemistry have revealed a large variety of compounds to be responsible for both flavor and/or aroma. In roasted foods nitrogenous heterocycles, pyrazines, have been implicated to be of particular organoleptic significance. Staudinger and Reichstein (1), were the first to report pyrazines in foods. A recent review by Maga and Sizer (2), lists seventy different pyrazines which have been identified in a variety of foods.

It has been proposed by several investigators, Dawes and Edwards (3), Rizzi (4), Mason et al. (5), and Wang et al. (6), that pyrazines are formed via a thermal cleavage of sugars into dicarbonyl fragments which undergo further reactions with α-amino acids, equation 1.



(1)

Although this mechanism involves the formation of dihydropyrazines as intermediates, their presence in foods has never been experimentally established. The difficulty of detecting dihydropyrazines has been

attributed by Bondarovich et al. (7) and by Juneja and Odell (8) to the relative instability of these compounds and the harsh treatments used to isolate basic compounds from foods. These methods, as reviewed by Maga and Sizer (2), generally involve steam distillation followed by concentration of the condensate, and fractionation using acidic and basic reagents. The basic fraction is then extracted with solvents prior to gas-liquid chromatographic analysis. In addition, Bondarovich et al. (7) have reported that some dihydropyrazines are unstable under certain gas-liquid chromatographic conditions. Thus, even though the reaction mechanism dictates the intermediate formation of dihydropyrazines, their detection would be rather problematic.

The lack of evidence for the presence of dihydropyrazines apparently has resulted in a relative absence of investigation into the chemical and biological characteristics of the compounds. Bondarovich et al. (7) and others have reported that dihydropyrazines have a roasted or nut-like aroma. Flament and Stoll (9) have reported some physical studies on certain alkylated dihydropyrazines. Juneja and Odell (8) have investigated the reactivity of alkylated dihydropyrazines toward certain dienophiles in an attempt to develop selective trapping agents for their detection. There have been no reports on the biological activity of the dihydropyrazines.

This paper reports the synthesis and chemical characterization of 2,3-dimethyl-5,6-dihydropyrazine. Similar studies have been reported earlier by Flament and Stoll (9). The isolation of urinary metabolites of 2,3-dimethyl-5,6-dihydropyrazine will also be discussed.

CHAPTER II

MATERIALS AND METHODS

Materials

Ammonium formate and anhydrous diethyl ether were purchased from Fisher Scientific Co., Fair Lawn, New Jersey. Ethylenediamine was obtained from Matheson, Coleman and Bell, Norwood, Ohio. 2,3-butanedione was purchased from Eastman Organic Chemicals, Rochester, New York. Carbowax and Gas Chrome Q were obtained from Applied Science Laboratories, Inc., State College, Pennsylvania. Sephadex G-10, Sephadex G-15, DEAE-Sephadex A-25, and CM-Sephadex C-25 were purchased from Pharmacia Fine Chemicals, Inc., Piscataway, New Jersey. CD-1 Strain, white, male mice were obtained from Charles River. 2,3-^{14}C-2,3-dimethyl-5,6-dihydropyrazine was a gift from Mr. Ralph Reed, Department of Biochemistry, Oklahoma State University.

Synthesis of 2,3-Dimethyl-5,6-Dihydropyrazine

The synthesis of 2,3-dimethyl-5,6-dihydropyrazine was based on the procedure described by Flament and Stoll (9) involving the condensation of ethylenediamine with 2,3-butanedione. The reaction was conducted in a one liter round bottom flask with three ground glass necks. In one neck was placed a 250 ml dropping funnel. The middle neck was fitted with a ground glass stirring rod and sleeve assembly which was connected

to a stirring motor. The third neck was connected to a 12 inch reflux condenser fitted with a tap water cooling jacket. The reaction vessel was mounted in an ice-water bath.

In the synthesis, 0.25 mole of ethylenediamine in 150 ml of ether was transferred to the cooled (0°) reaction flask. A solution of 0.25 mole of 2,3-butanedione in 215 ml of ether was then added dropwise through the dropping funnel while stirring the reaction mixture. A light tan precipitate formed as the 2,3-butanedione was added. The 2,3-butanedione was added dropwise over a period of one hour and the reaction was allowed to continue for 30 minutes after the last addition. The ice-water bath was then removed and the reaction flask warmed to 25°. The solution was then allowed to stand at 25° until the light tan precipitate had dissolved, forming a dark yellow solution.

The aqueous and ether layers were separated and the aqueous layer was extracted four times with 20 ml of diethyl ether. The ether extracts were combined with the original ether layer. The volume of the ether solution was reduced by removing most of the ether on a rotary evaporator at 25°.

Purification of 2,3-Dimethyl-5,6-Dihydropyrazine

The purification of 2,3-dimethyl-5,6-dihydropyrazine was effected using fractional distillation under reduced pressure. The reaction mixture was transferred to a 250 ml round bottom distillation flask with a ground glass neck. The neck was fitted with a silver-mirrored, tap water jacketted, three inch reflux condenser with a side arm and a ground glass joint fitted with a thermometer. The side arm was connected to a water cooled four inch condenser with a vacuum nipple

connected to a water aspirator. The condenser was connected to a collection apparatus fitted with three ground glass male connections to which 50 ml round bottom flasks with ground glass necks were fitted. The distillation flask was mounted in a heating mantel controlled by a voltage regulator.

The reaction mixture was then fractionally distilled under vacuum, 12 mm Hg. The second fraction, which distilled at 60°, was collected and saved. Other fractions were discarded and a dark brown gummy residue was noted in the distillation flask. The purified compound was stored under nitrogen.

Physical Characterization of 2,3-Dimethyl-5,6-Dihydropyrazine

Gas-Liquid Chromatography

A Perkin-Elmer Model 990 gas chromatograph fitted with a flame-ionization detector was used to determine the purity of the synthesized 2,3-dimethyl-5,6-dihydropyrazine. The column was a 1/4 inch x 6 foot glass column packed with five percent (w/w) Carbowax 20M on Gas Chrome Q (100/120 mesh). The column was operated isothermally at 135° using nitrogen as the carrier gas. The injector temperature was 175° and the detector temperature was 200°. One ul samples were injected neat into the gas chromatograph.

Infrared Spectroscopy

The infrared spectrum of the synthesized 2,3-dimethyl-5,6-dihydropyrazine was obtained on a Perkin Elmer Model 457 grating infrared

spectrophotometer. The compound was scanned neat as a 0.028 mm thick film between two sodium chloride plates.

Magnetic Resonance Spectroscopy

The nuclear magnetic resonance spectrum of 2,3-dimethyl-5,6-dihydropyrazine was obtained on a Varian 60 megacycle instrument. The sample was scanned neat at 25°.

Mass Spectrometry

The mass spectrum of synthesized 2,3-dimethyl-5,6-dihydropyrazine was determined using a combination gas chromatograph-mass spectrometer. The instrument used was a LKB 9000 mass spectrometer.

Ultraviolet Spectroscopy

The ultraviolet spectrum of 2,3-dimethyl-5,6-dihydropyrazine was obtained on a Hitachi Perkin-Elmer Model 124 double-beam spectrophotometer. A 0.42 M solution of the sample in water was scanned from 360 nm to 190 nm using de-ionized water in the reference beam.

Ionization Constants

The ionization constants of 2,3-dimethyl-5,6-dihydropyrazine were determined using a titrimetric technique. A Radiometer Model PHM25 pH meter was used to follow the titration. Fifty ml of a 0.05 M solution of the compound in water was titrated with 0.18 M hydrochloric acid. Corrections were made for the titration of 50 ml of de-ionized water with 0.18 M hydrochloric acid and the volume of titrant used was converted to milliequivalents. The first derivative of the titration

curve was computed to determine the change in buffer capacity as a function of a change in pH ($\Delta B/\Delta pH$). The peaks of the $\Delta B/\Delta pH$ curve were defined as the ionization constants.

Biological Activity

Toxicity

The toxicity of 2,3-dimethyl-5,6-dihydropyrazine was determined by the method of Reed and Muench (10). Different dilutions of the compound in 0.9 percent saline solution were made and 0.25 ml was injected into the test animals. Four white male mice of approximately 25 g were injected intraperitoneally with 0.25 ml of the test sample at each dilution. All deaths occured within three hours. No postmortem examinations were performed due to time limitations.

Daily Urinary Excretion of ^{14}C Labeled Metabolites

Four white male mice were each injected intraperitoneally with five mg of 2,3-^{14}C-2,3-dimethyl-5,6-dihydropyrazine, 53,400 dpm/mg, in 0.25 ml of 0.9 percent saline solution. The mice were then placed in a metabolic cage mounted above a large funnel fitted with a collection flask. The collection flask was suspended in an ice-water bath. During the five day test period, the mice were allowed free access to both food and water.

The urine was collected at 24 hour intervals and centrifuged at 10,000 x g for 15 minutes. The pellet and supernatant were separated and the pellet resuspended in water and centrifuged at 10,000 x g for 15 minutes. The two supernatants were combined and diluted to 250 ml.

One ml aliquots of the dilution were transferred to a scintillation vial and diluted with 10 ml of liquid scintillation solution prepared according to the method of Bray (11). The samples were then assayed for radiation on a Packard Liquid Scintillation Spectrophotometer Model 3320. Corrections were made for background, dilutions, and instrument efficiency.

Isolation of Urinary Metabolites

Ten white male mice were each injected intraperitoneally with five mg of 2,3-^{14}C-2,3-dimethyl-5,6-dihydropyrazine in 0.25 ml of 0.9 percent saline solution. The mice were then placed in a metabolic cage as above. Since it was determined that most of the ^{14}C label was excreted in the first 24 hours, sampling was not continued beyond this time. The 24 hour urine sample was then centrifuged and the pellet washed as above. The sample was then dried by lyophilization.

The urinary metabolites of 2,3-dimethyl-5,6-dihydropyrazine were then isolated using chromatographic techniques. The samples were monitored for radiation by assaying 0.1 ml in a liquid scintillation system as above. The samples were also followed spectrophotometrically by measuring the absorption at 260 nm.

Anion Exchange Chromatography

The dried urine sample was diluted to five ml with 0.04 M ammonium formate. The sample was then placed on top of an anion exchange column and eluted with buffer. Five ml fractions were collected and monitored as above.

The column was a 1.0 cm x 100 cm column packed with DEAE-Sephadex

A-25 formate form. The eluting buffer was 0.04 M ammonium formate solution, pH 6.47.

Cation Exchange Chromatography

The ^{14}C labeled fraction from the anion exchange chromatography was dried by lyophilization. The sample was then diluted to five ml in 0.04 M ammonium formate solution. The sample was then placed on top of a cation exchange column and eluted by increasing the ammonium formate concentration. Four ml fractions were collected and monitored as above.

The column was a 1.5 cm x 60 cm column packed with CM-Sephadex C-25 ammonium form. The column was washed with 50 ml of 0.04 M ammonium formate, pH 6.47. A linear elution gradient was then initiated. A volume of 250 ml of 0.04 M ammonium formate, pH 6.47, was placed in a mixing chamber which was attached to a reservoir containing 250 ml of 1.5 M ammonium formate, pH 6.47.

Molecular Seive Chromatography

Sephadex G-15. ^{14}C labeled fractions, designated 2 and 3 from the cation exchange chromatography were dried by lyophilization. They were diluted to five ml with water and placed on 1.5 cm x 100 cm Sephadex G-15 columns. The samples were eluted with water and two ml fractions were collected. The samples were monitored as above.

Sephadex G-10. ^{14}C labeled fractions, designated 2a and 2b from the Sephadex G-15 column and 1 from the cation exchange column, were dried by lyophilization. They were diluted to five ml with water and placed on 1.5 cm x 100 cm Sephadex G-10 columns. The samples were

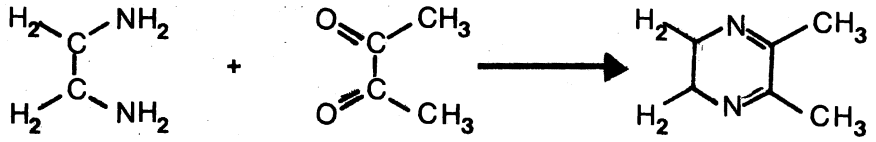
eluted with water and two ml fractions collected. The samples were monitored as above.

CHAPTER III

RESULTS AND DISCUSSION

Synthesis and Purification of 2,3-Dimethyl-5,6-Dihydropyrazine

The synthesis of 2,3-dimethyl-5,6-dihydropyrazine (III) was accomplished by condensing ethylenediamine (I) with 2,3-butanedione (II) at 0°, equation 2. The concentrated reaction mixture was dark yellow.



(I) (II) (III) (2)

Fractional distillation under reduced pressure was found to be a very satisfactory method for purification. This procedure resulted in 2,3-dimethyl-5,6-dihydropyrazine that was greater than 99 percent pure, as estimated by the gas-liquid chromatographic pattern of Figure 1. The purified compound was a light yellow. The synthetic yield, through purification, was 13.5 ml which represents a 47 percent yield. This is in good agreement with Flament and Stoll (9), who reported a yield of 50 percent.

Physical Characterization of 2,3-Dimethyl-5,6-Dihydropyrazine

The physical characterization of 2,3-dimethyl-5,6-dihydropyrazine

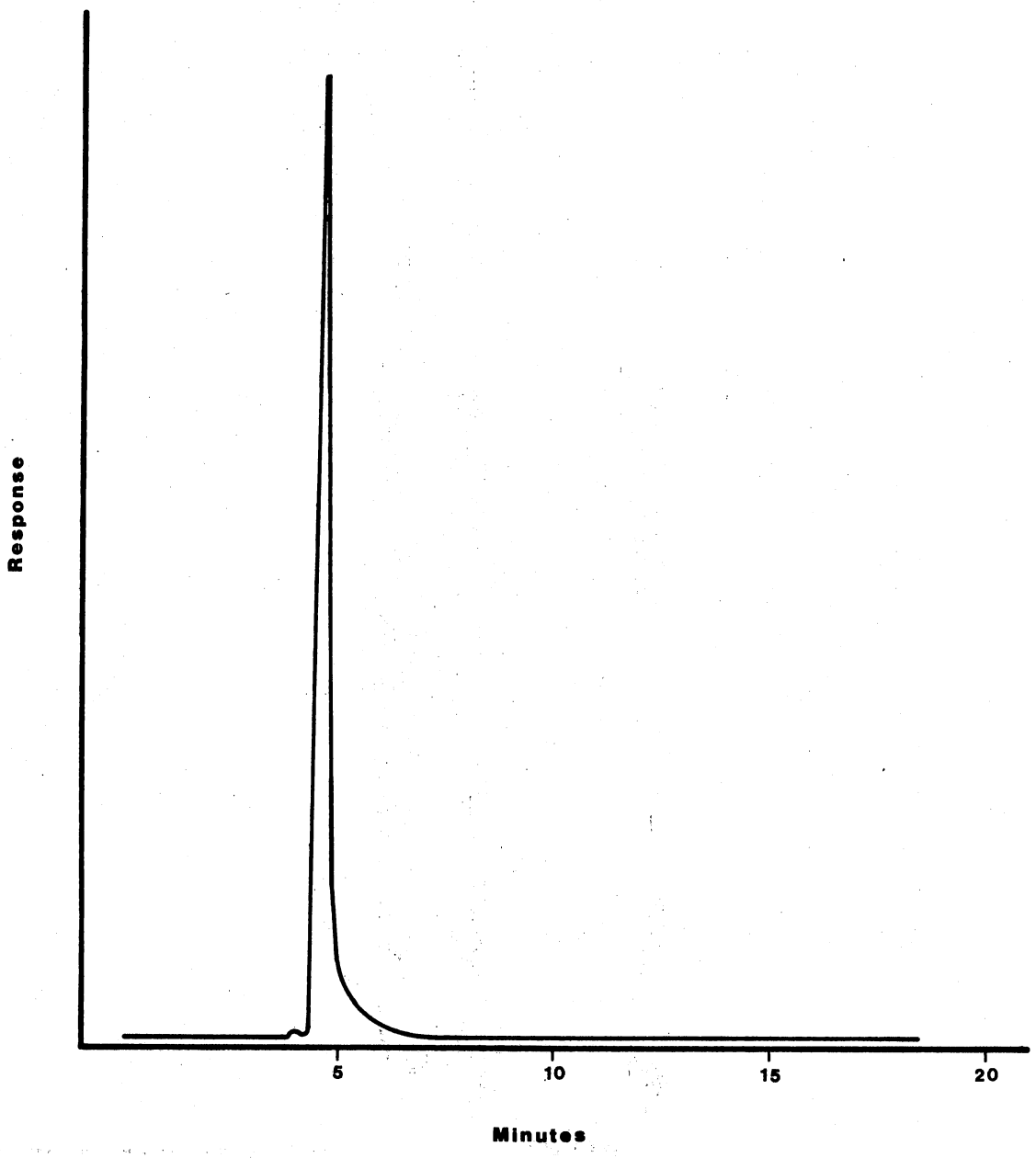


Figure 1. Gas-liquid Chromatogram of Synthetic 2,3-Dimethyl-5,6-Dihydropyrazine

was considered necessary for two main reasons. The first reason was to verify the structure of the synthesized 2,3-dimethyl-5,6-dihydropyrazine. The second reason was to provide a basis for comparison for future studies concerned with characterizing the metabolites of 2,3-dimethyl-5,6-dihydropyrazine. Although Flament and Stoll (9) provide a short discussion of some of these physical properties, the actual data is not provided in the form of usable figures.

The infrared spectrum of 2,3-dimethyl-5,6-dihydropyrazine is shown in Figure 2. The bands at 1652-1647, 1597-1590, 951-925, 896-883, and 662-645 cm^{-1} were reported by Flament and Stoll (9).

The nuclear magnetic resonance spectrum of 2,3-dimethyl-5,6-dihydropyrazine (Figure 3) indicates the presence of only two types of hydrogen atoms. The large peak at 2.05 ppm is consistent with methyl hydrogens. The second peak at 3.19 ppm represents the ring hydrogens. The measured ratio of these two peaks is 6.0 to 4.0 which is the predicted ratio for the compound.

The mass spectral pattern of 2,3-dimethyl-5,6-dihydropyrazine is shown in Figure 4. The peak at m/e 110 represents the molecular ion of the compound. The identification of the other m/e peaks would require high resolution mass spectrometry techniques.

The ultraviolet spectrum of 2,3-dimethyl-5,6-dihydropyrazine from 190 nm to 360 nm is shown in Figure 5. The two largest peaks occur at 200 nm and 225 nm. A smaller peak is observed at 320 nm. Log ε for the peak at 200 nm is 3.45 and that for the 225 nm peak is 3.29.

The ionization constants of 2,3-dimethyl-5,6-dihydropyrazine were determined (Figure 6). The first ionization constant is 5.9 and the second occurs at 1.3. These are considerably higher than those found

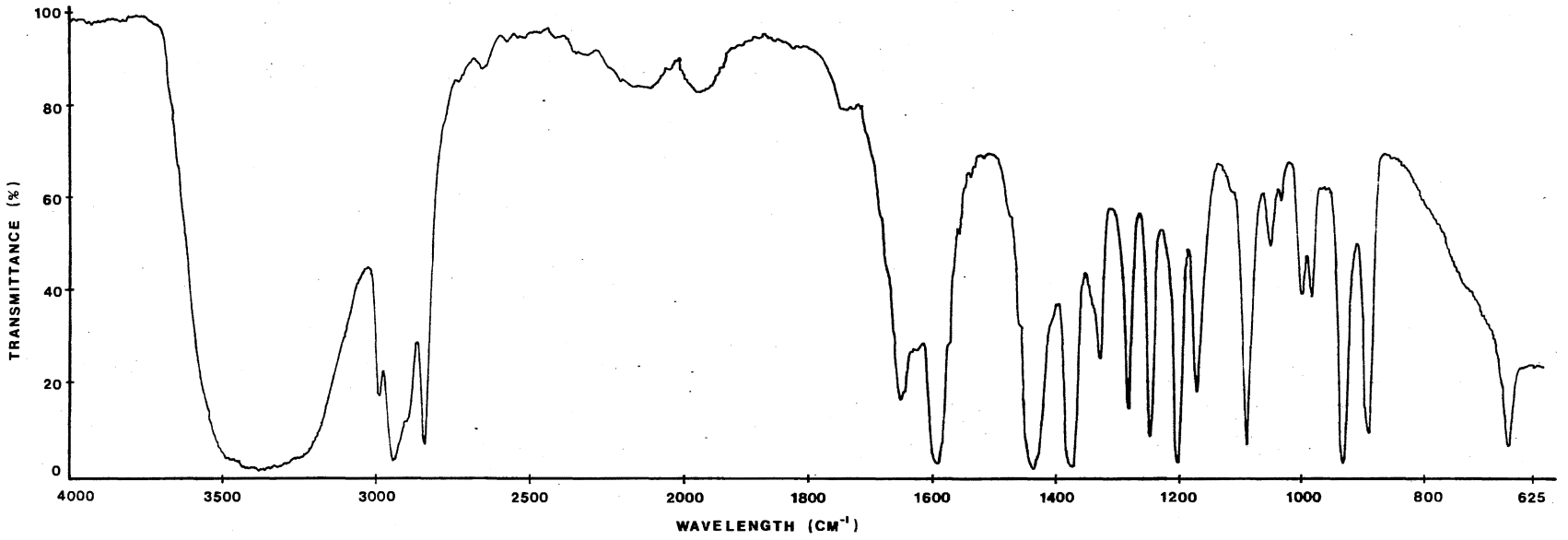


Figure 2. Infrared Spectrum of 2,3-Dimethyl-5,6-Dihydropyrazine

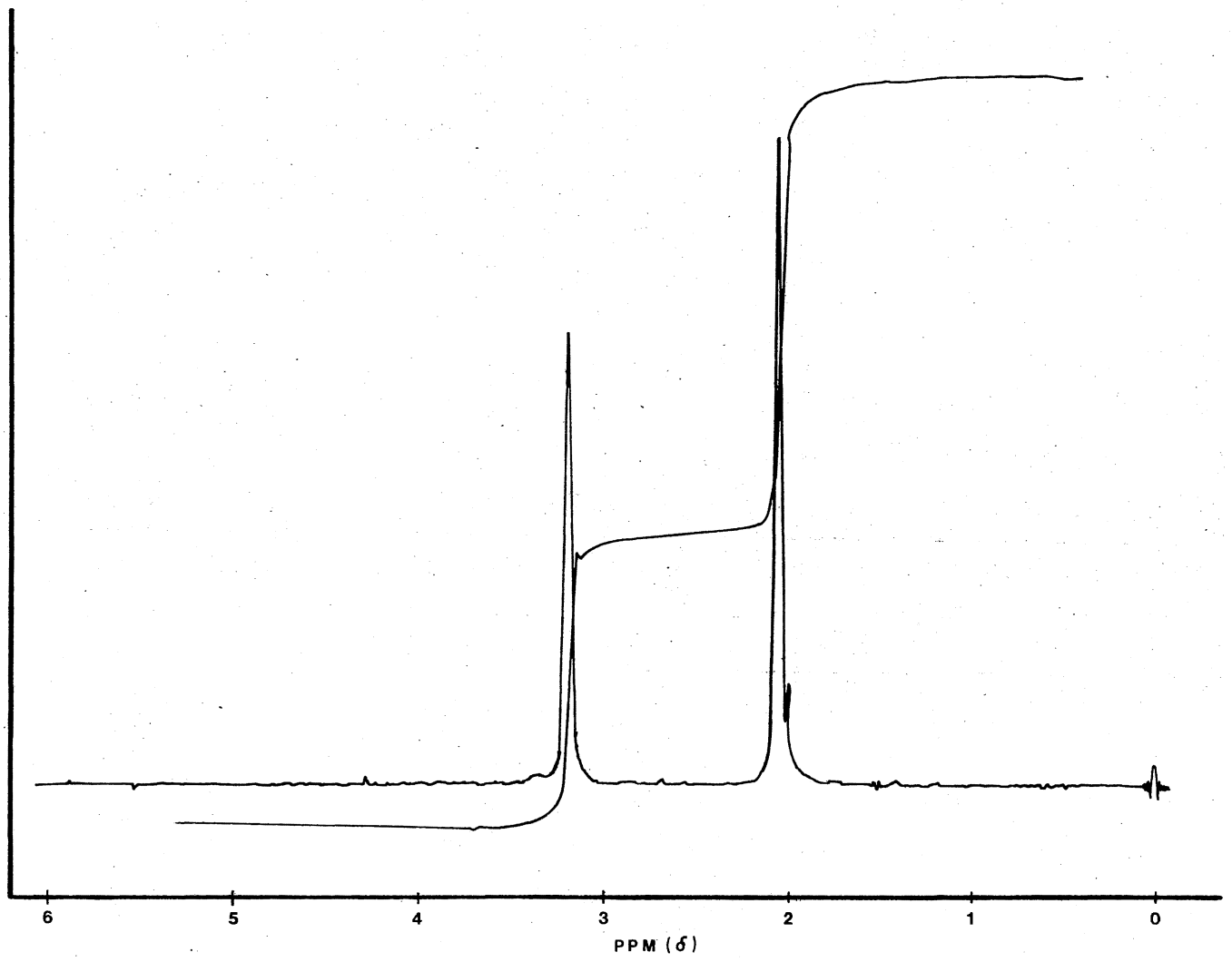


Figure 3. Nuclear Magnetic Resonance Spectrum of 2,3-Dimethyl-5,6-Dihydropyrazine

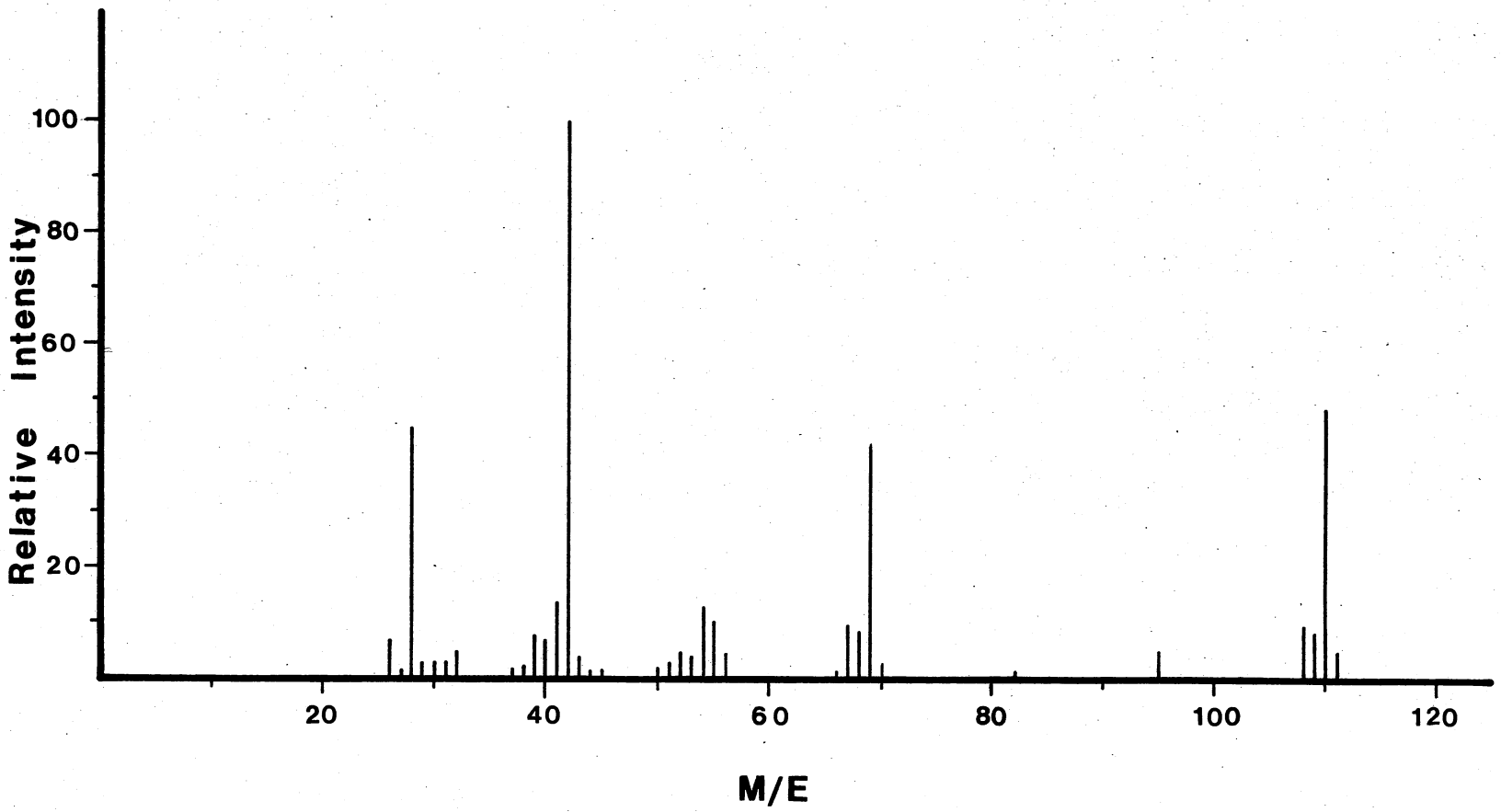


Figure 4. Mass Spectrum of 2,3-Dimethyl-5,6-Dihydropyrazine

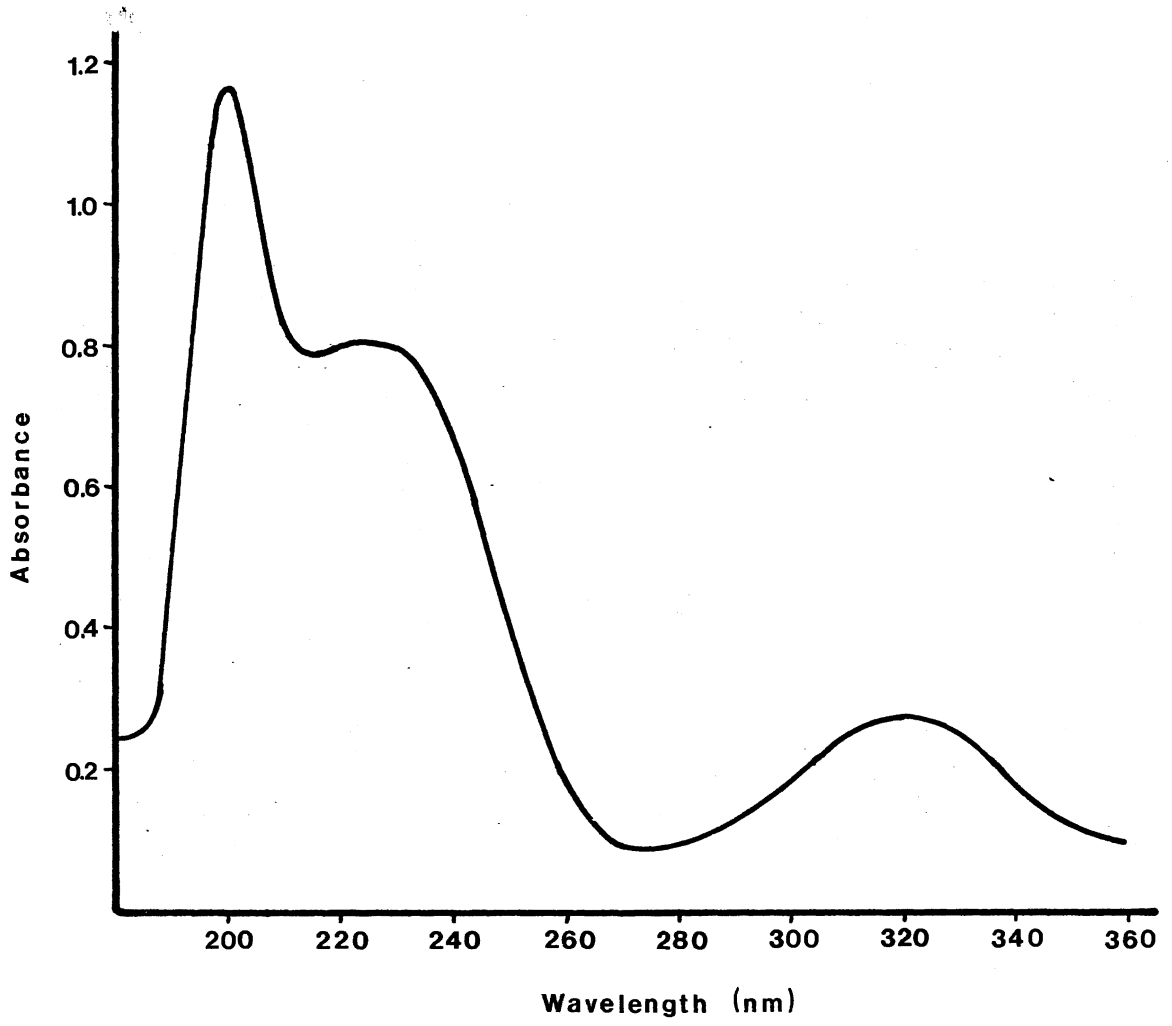


Figure 5. Ultraviolet Spectrum of 2,3-Dimethyl-5,6-Dihydropyrazine

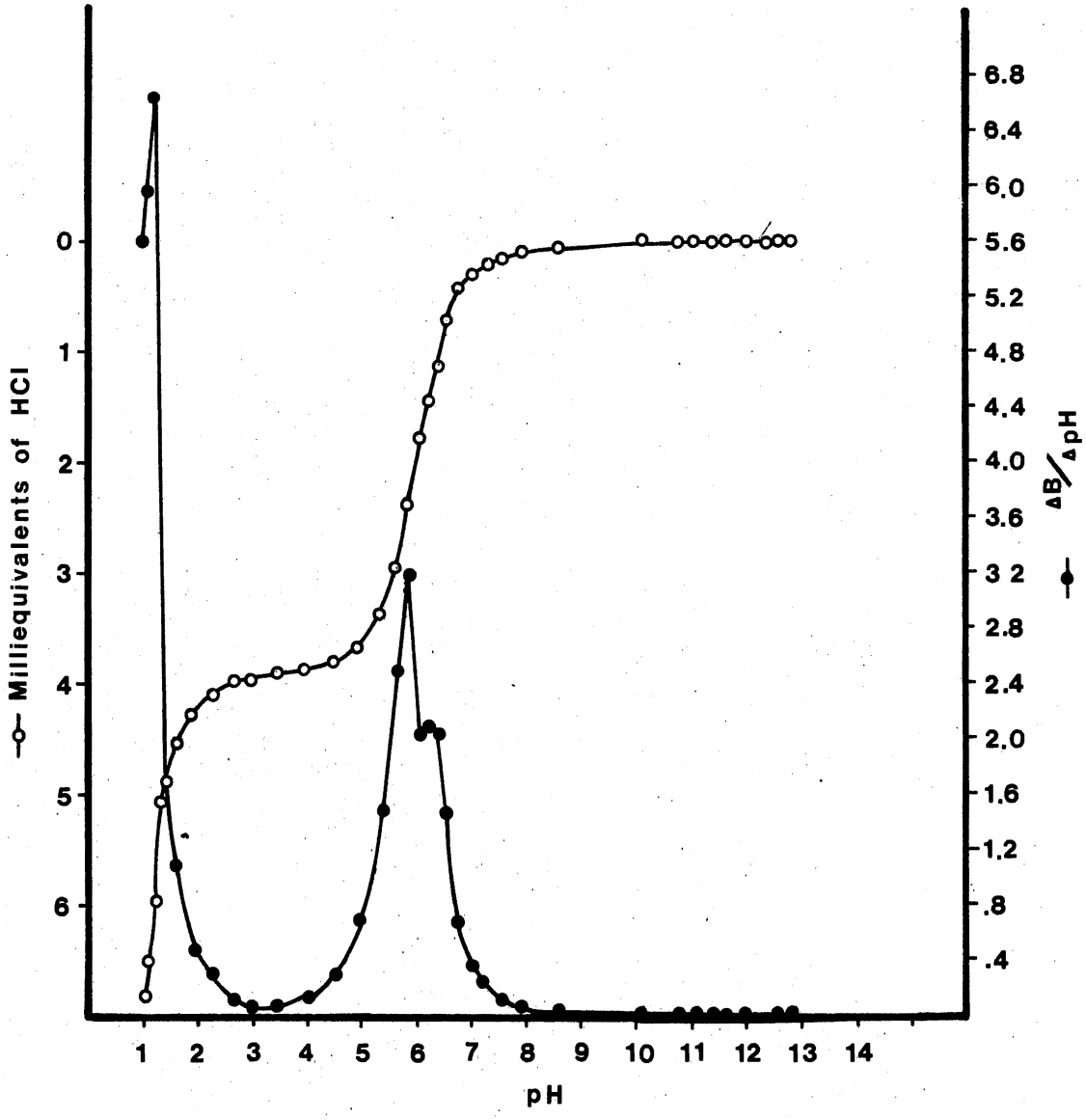


Figure 6. Titration of 2,3-Dimethyl-5,6-Dihydropyrazine With HCl

for pyrazines which are approximately 0.65 and -5.8 respectively. This large difference is expected since the lack of aromatization in the 2,3-dimethyl-5,6-dihydropyrazine generates a less stable ring system than is present in the pyrazines.

Biological Activity of 2,3-Dimethyl-5,6-Dihydropyrazine

During early studies with 2,3-dimethyl-5,6-dihydropyrazine, it was noted that contact of the compound with the skin resulted in a rapid browning of the epidermal tissue. This dark brown stain was extremely difficult to remove. It was decided that a compound which binds this tightly should be investigated with respect to both toxicity and elimination from the body.

The toxicity, in terms of LD_{50}, was determined to be 0.36 mg/g body weight when injected intraperitoneally into white male mice (Figure 7). The LD_{50} of 2,3-dimethylpyrazine has been reported to be 1.39 mg/g body weight in mice. Extreme muscular contractions began immediately upon injection at the higher concentrations. A hypnotic effect was observed at lower concentrations which lasted up to three hours. Doses in the range of the LD_{50} resulted in muscular spasms in the animals which sometimes lasted up to 1.5 hours but did not always result in death. Deaths occurred within three hours.

Daily urinary excretion of metabolites was monitored by using ^{14}C labeled 2,3-dimethyl-5,6-dihydropyrazine. The percent dose recovered was computed as the percentage of the total label injected into four white male mice (Figure 8). The largest recovery of label in the urine was 43 percent and occurred during the first 24 hours. This compares

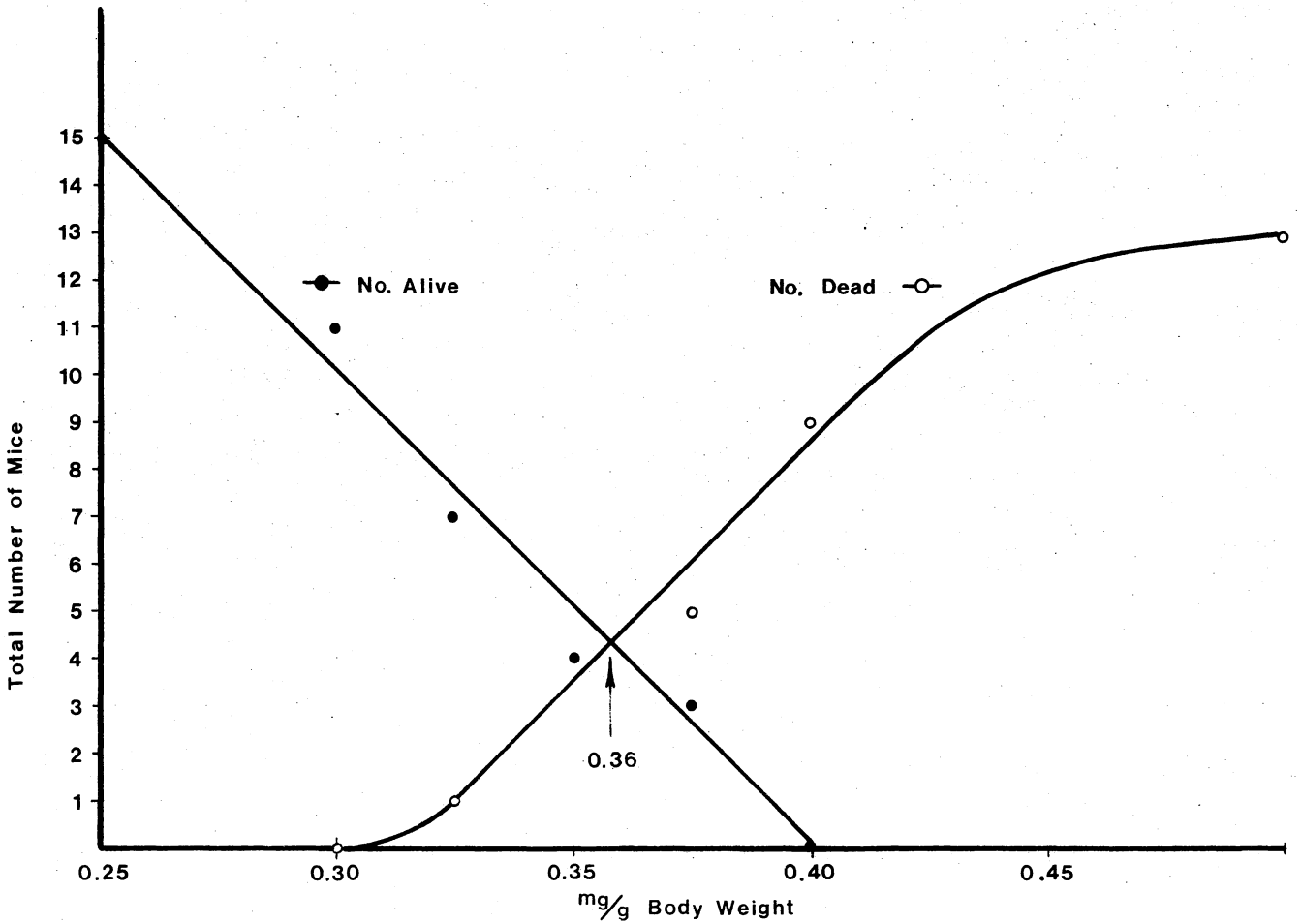


Figure 7. LD_{50} of 2,3-Dimethyl-5,6-Dihydropyrazine for Mice

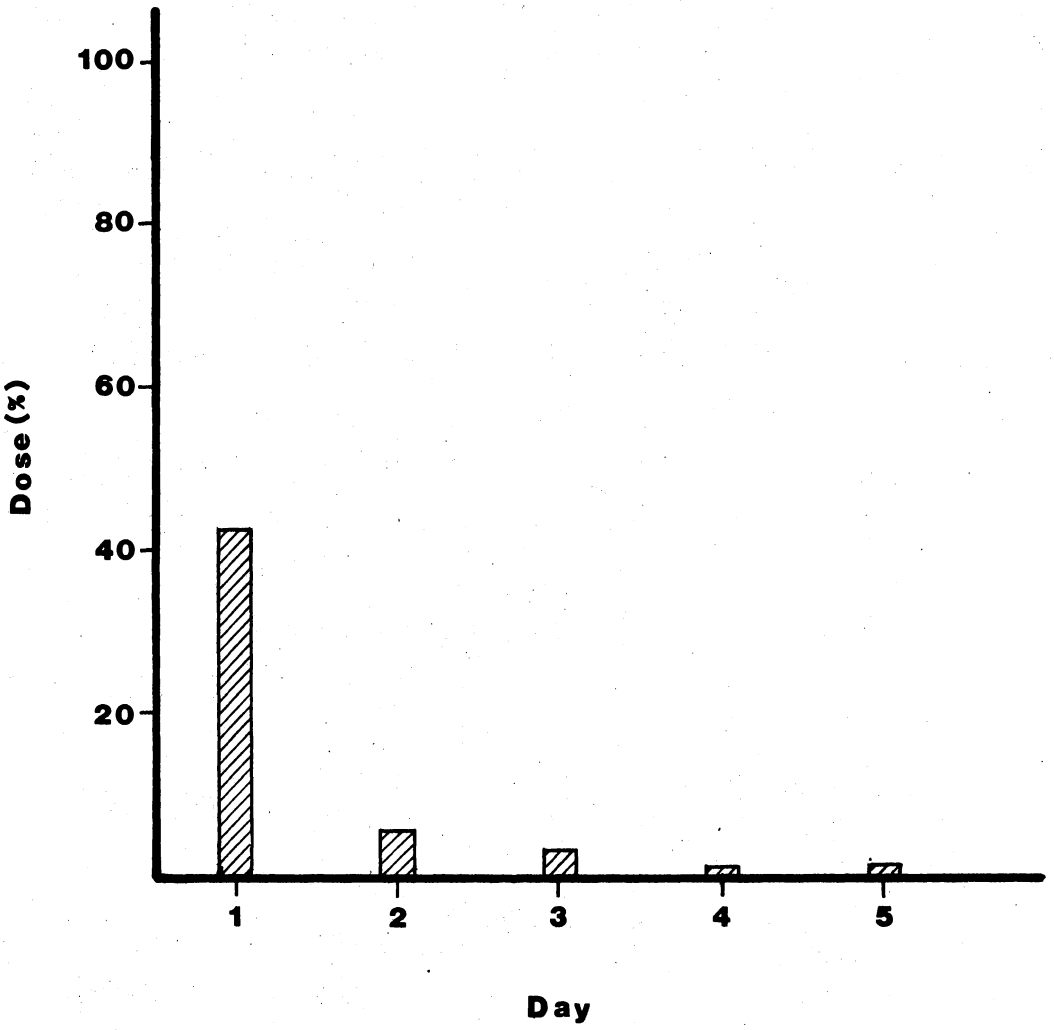


Figure 8. Daily Excretion of ^{14}C Labeled Metabolites in Urine of Mice

with 84 percent of the label being excreted in the urine of rats in 24 hours when 2,3-^{14}C-2,3-dimethylpyrazine is administered as reported by Elgin (12). The total recovery of label during the five day period was 55 percent. Whether the remainder of the radioactivity was eliminated via the respired gases or in the feces is unknown. It can be postulated that some of the injected compound may have become associated with some of the various tissues with which it came into contact.

Isolation of Urinary Metabolites

The initial separation of the urinary metabolites of 2,3-dimethylpyrazine, as performed by Reed (13), involves their elution from a Dowex 1 x 8 formate form column using low pH and high salt concentrations. It was thought that the metabolites of 2,3-dimethyl-5,6-dihydropyrazine might be similar enough to also utilize anion exchange chromatography for their initial separation. Since 2,3-dimethyl-5,6-dihydropyrazine is chemically less stable than the 2,3-dimethylpyrazine, it was decided that a milder procedure was needed. A DEAE-Sephadex A-25 formate form column was selected and the elution buffer was 0.04 M ammonium formate, pH 6.47.

The DEAE-Sephadex A-25 column did not separate the urinary metabolites of 2,3-dimethyl-5,6-dihydropyrazine, since all of the ^{14}C label eluted in the void volume, designated fraction A (Figure 9). It was effective, however, in removing a large amount of the compounds present in the urine of the mice. This procedure was therefore utilized as the initial step.

Three controls were run using identical conditions to the DEAE-Sephadex system. The first control was half of a 24 hour urine sample

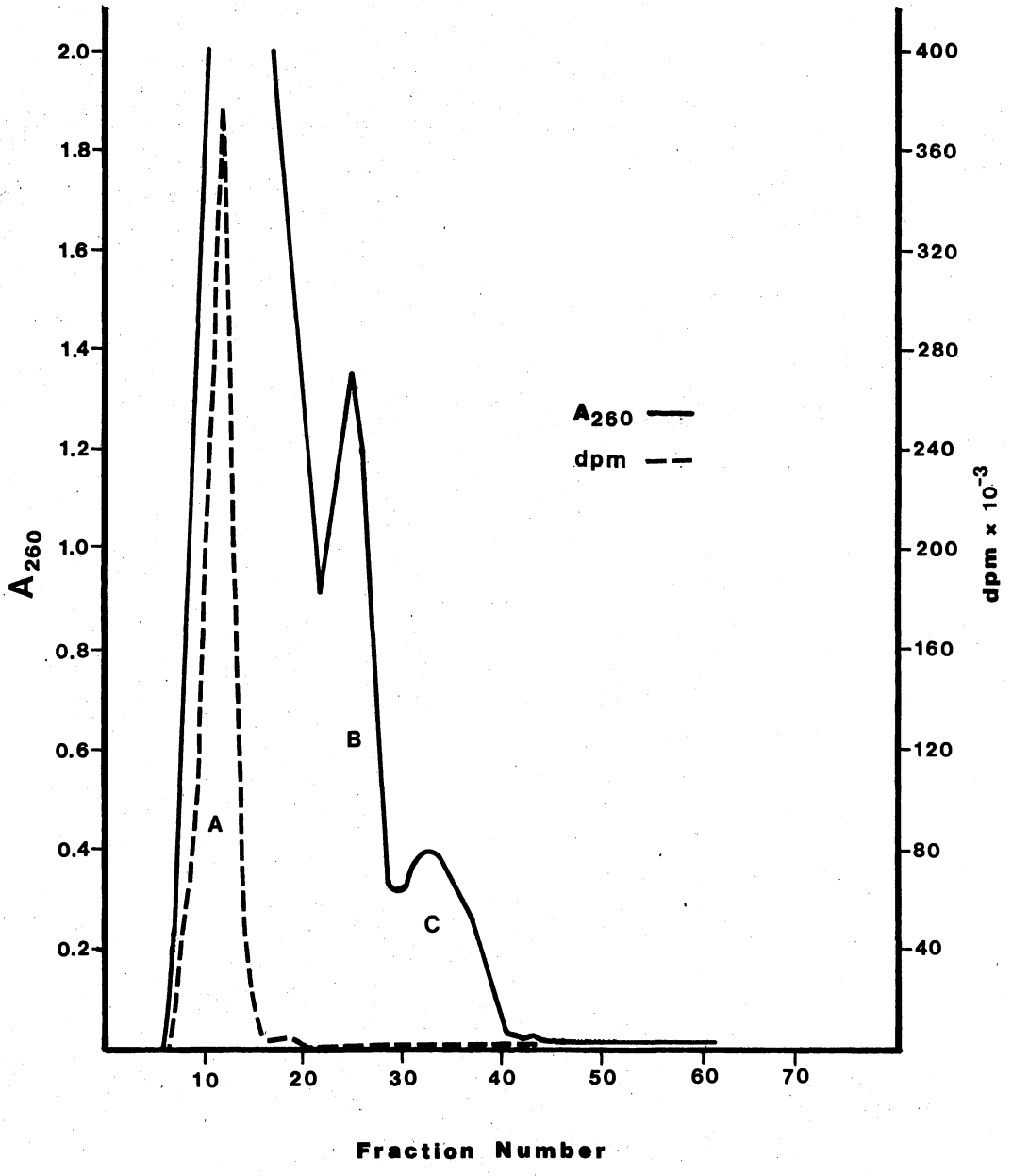


Figure 9. DEAE-Sephadex Chromatogram of ^{14}C Labeled Metabolites

from one mouse (Figure 10). The second was five mg of 2,3-dimethyl-5,6-dihydropyrazine (Figure 11). The third control was five mg of 2,3-^{14}C-2,3-dimethyl-5,6-dihydropyrazine incubated two hours at 25° with a 24 hour urine sample from one mouse (Figure 12).

Since the urinary metabolites did not separate on the anion exchange column, the decision was made to attempt the separation of fraction A, from the DEAE-Sephadex column, using a cation exchange column. The conditions of the separation were again kept mild. A weak cation exchange resin, CM-Sephadex C-25, was chosen and the separation achieved using an increasing ammonium formate gradient, maintaining the pH at 6.47.

The CM-Sephadex C-25 column resolved the urinary metabolites into three major ^{14}C labeled fractions (Figure 13). These three fractions were designated 1, 2, and 3 in order of their elution.

The two radioactive peaks obtained in the third control for the DEAE-Sephadex system (Figure 12) were combined and rechromatographed using the CM-Sephadex system. Three ^{14}C labeled fractions were obtained (Figure 14) but only one of these could be correlated with the pattern for the urinary metabolites of Figure 13.

The spectrophotometric and ^{14}C labeling pattern (Figure 13) indicated that the purification of the urinary metabolites was not complete and molecular seive chromatography was then attempted.

The ^{14}C labeled fraction 1 (Figure 13) was rechromatographed using a Sephadex G-10 column. Five ^{14}C labeled metabolites were now detected and designated 1a, 1b, 1c, 1d, and 1e (Figure 15). Purification was still not complete but time did not allow further efforts.

The ^{14}C labeled fraction 2 (Figure 13) was rechromatographed using

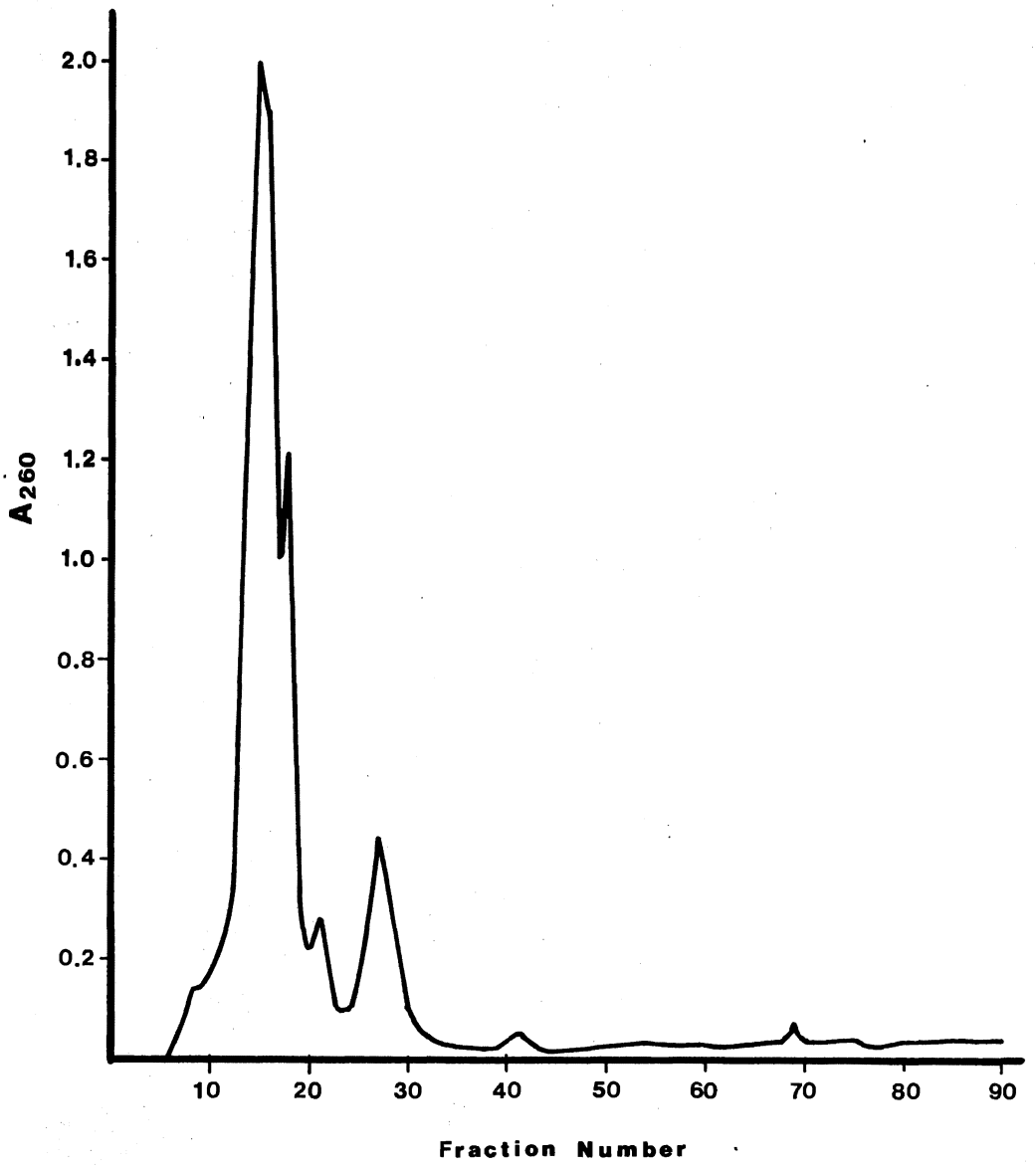


Figure 10. DEAE-Sephadex Chromatogram of Mouse Urine

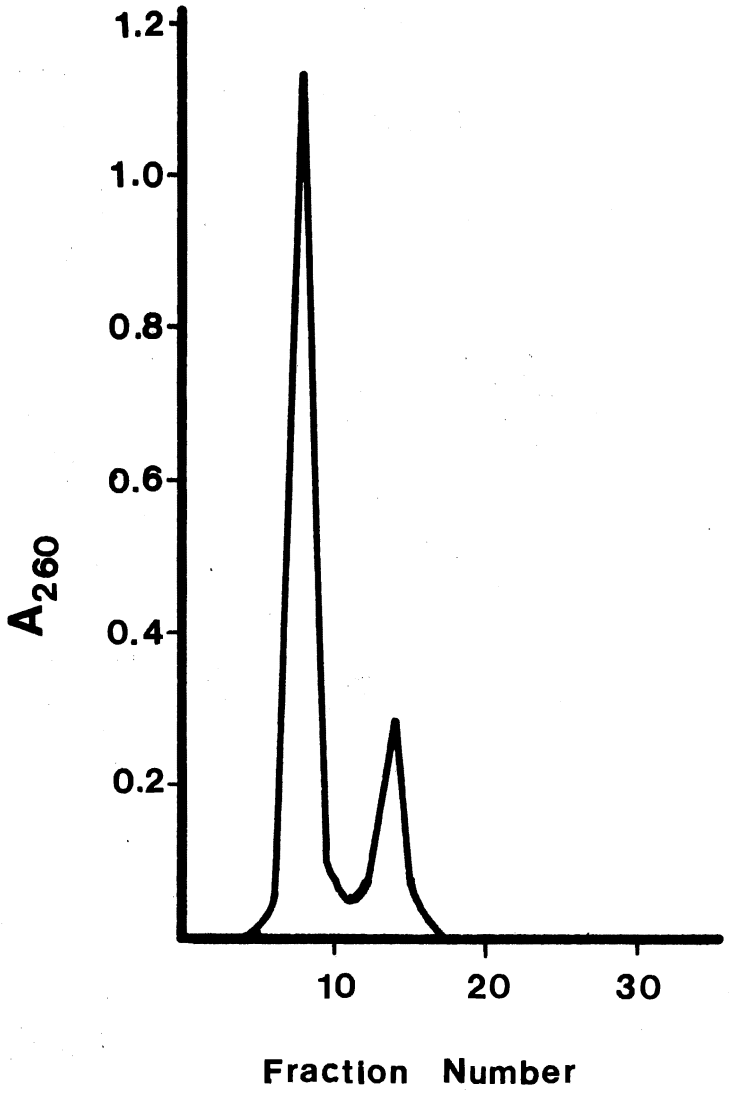


Figure 11. DEAE-Sephadex Chromatogram of 2,3-Dimethyl-5,6-Dihydropyrazine

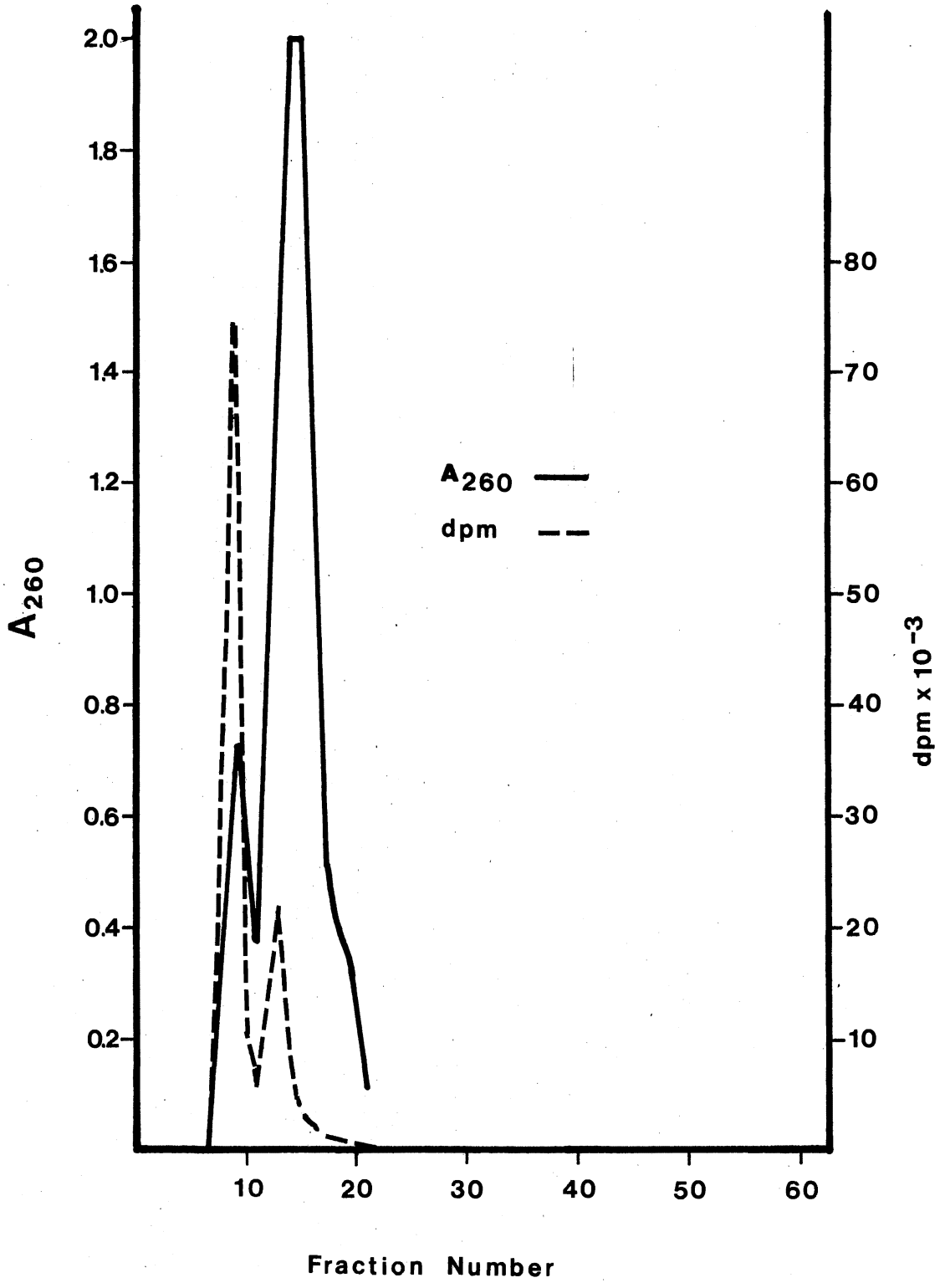


Figure 12. DEAE-Sephadex Chromatogram of 2,3-^{14}C-2,3-Dimethyl-5,6-Dihydropyrazine Plus Mouse Urine

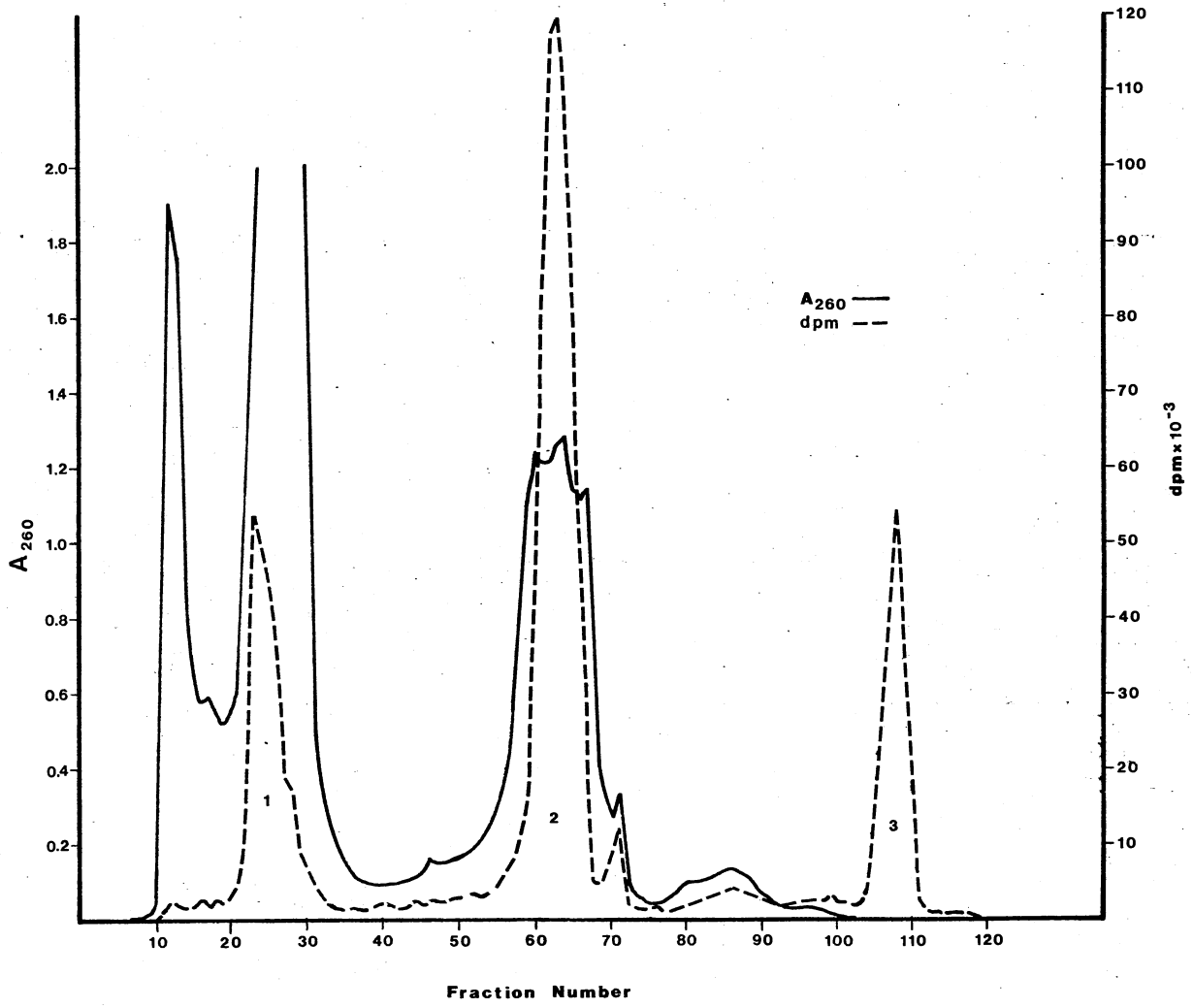


Figure 13. CM-Sephadex Chromatogram of Fraction A

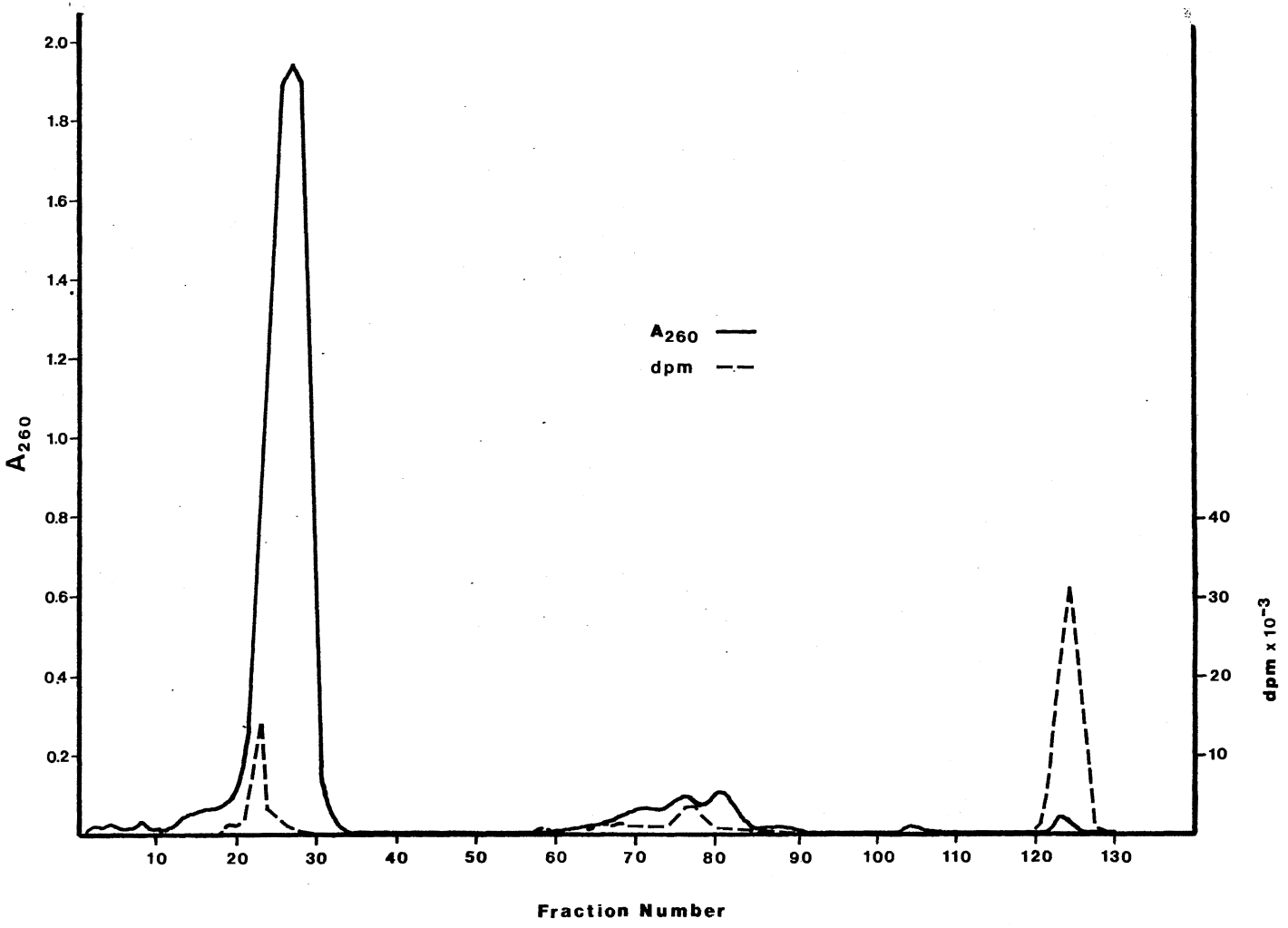


Figure 14. CM-Sephadex Chromatogram of 2,3-^{14}C-2,3-Dimethyl-5,6-Dihydropyrazine Plus Mouse Urine

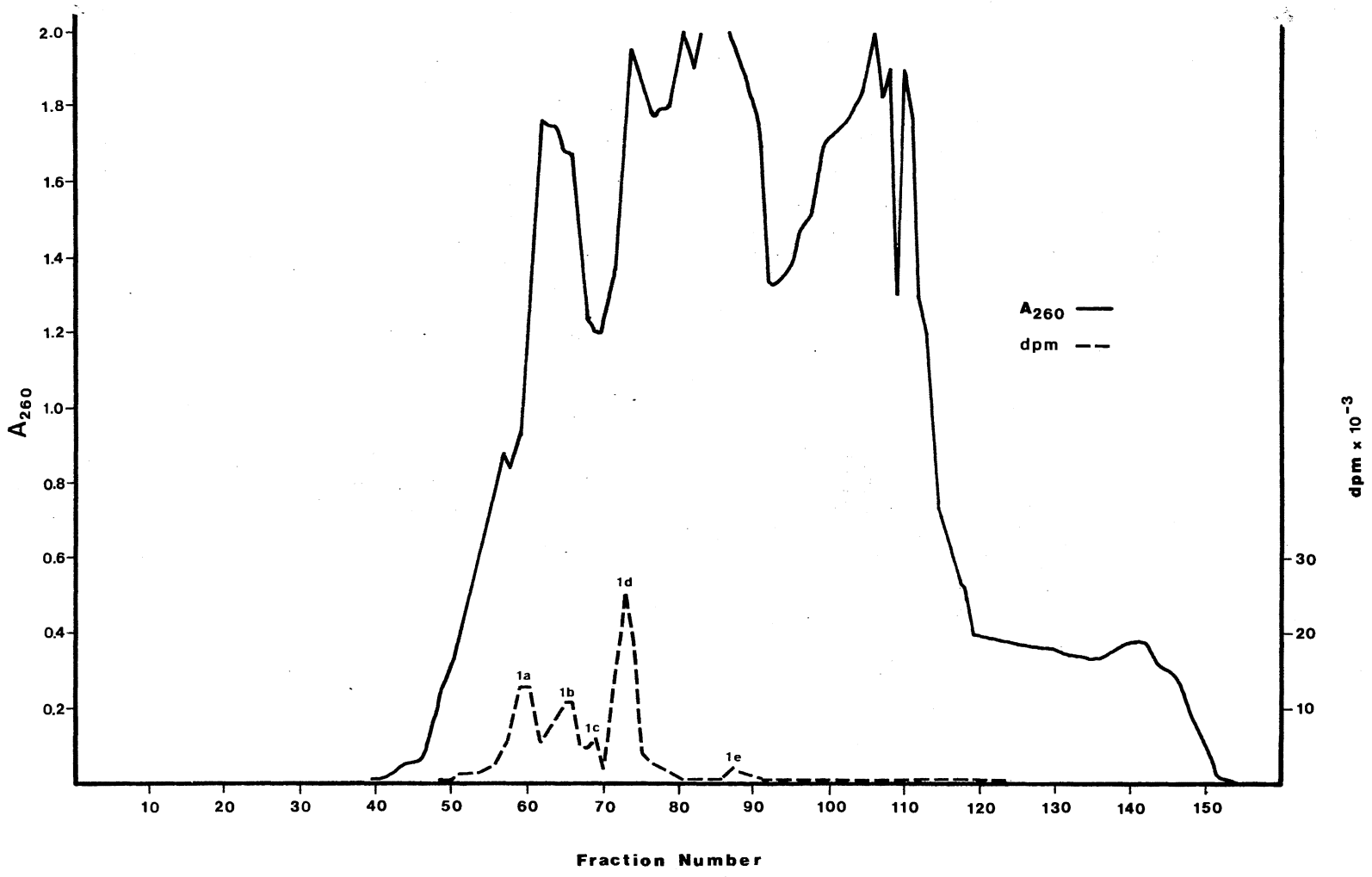


Figure 15. Sephadex G-10 Chromatogram of Fraction 1

a Sephadex G-15 column. Two ^{14}C labeled fractions were found and were designated 2a and 2b (Figure 16). Purification was still not satisfactory. Fractions 2a and 2b were rechromatographed on Sephadex G-10 columns (Figures 17 and 18).

The ^{14}C labeled fraction 3 (Figure 13) was found not to absorb light at either 260 nm or 280 nm. Fraction 3 was rechromatographed using a Sephadex G-15 column (Figure 19).

The isolation scheme for the urinary metabolites of 2,3-dimethyl-5,6-dihydropyrazine is shown in Figure 20. It is noted above that the purification of the metabolites is not yet complete. Reed (13) has had success with various thin-layer and paper chromatographic techniques in the isolation of the urinary metabolites of 2,3-dimethylpyrazine. It may be possible to use these techniques to further purify the metabolites of 2,3-dimethyl-5,6-dihydropyrazine.

Reed (13) has established that there are at least four urinary metabolites of 2,3-dimethylpyrazine in rats. This study indicates at least five minor and three major urinary metabolites of 2,3-dimethyl-5,6-dihydropyrazine in mice. Future studies might be concerned with the comparison of the metabolites of 2,3-dimethylpyrazine and 2,3-dimethyl-5,6-dihydropyrazine. The percentage of the injected ^{14}C label of each of the eight metabolites are shown in Table I.

Due to time limitations, no attempt has been made to characterize the metabolites. Further, the results that could be obtained at this stage of purity would be of little value. Future studies should complete the purification and the characterization of the metabolites. The localization of the metabolic processes would also be a useful

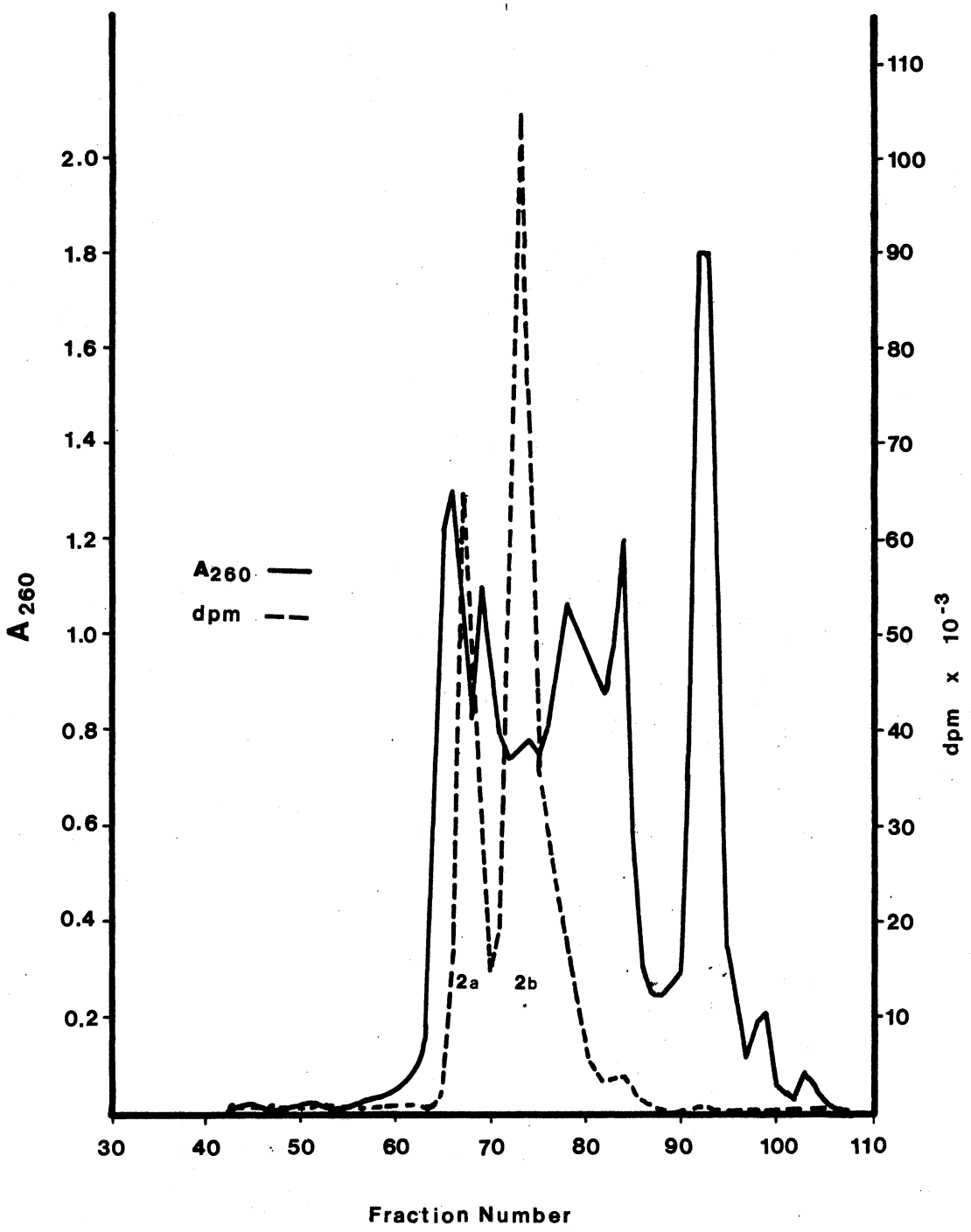


Figure 16. Sephadex G-15 Chromatogram of Fraction 2

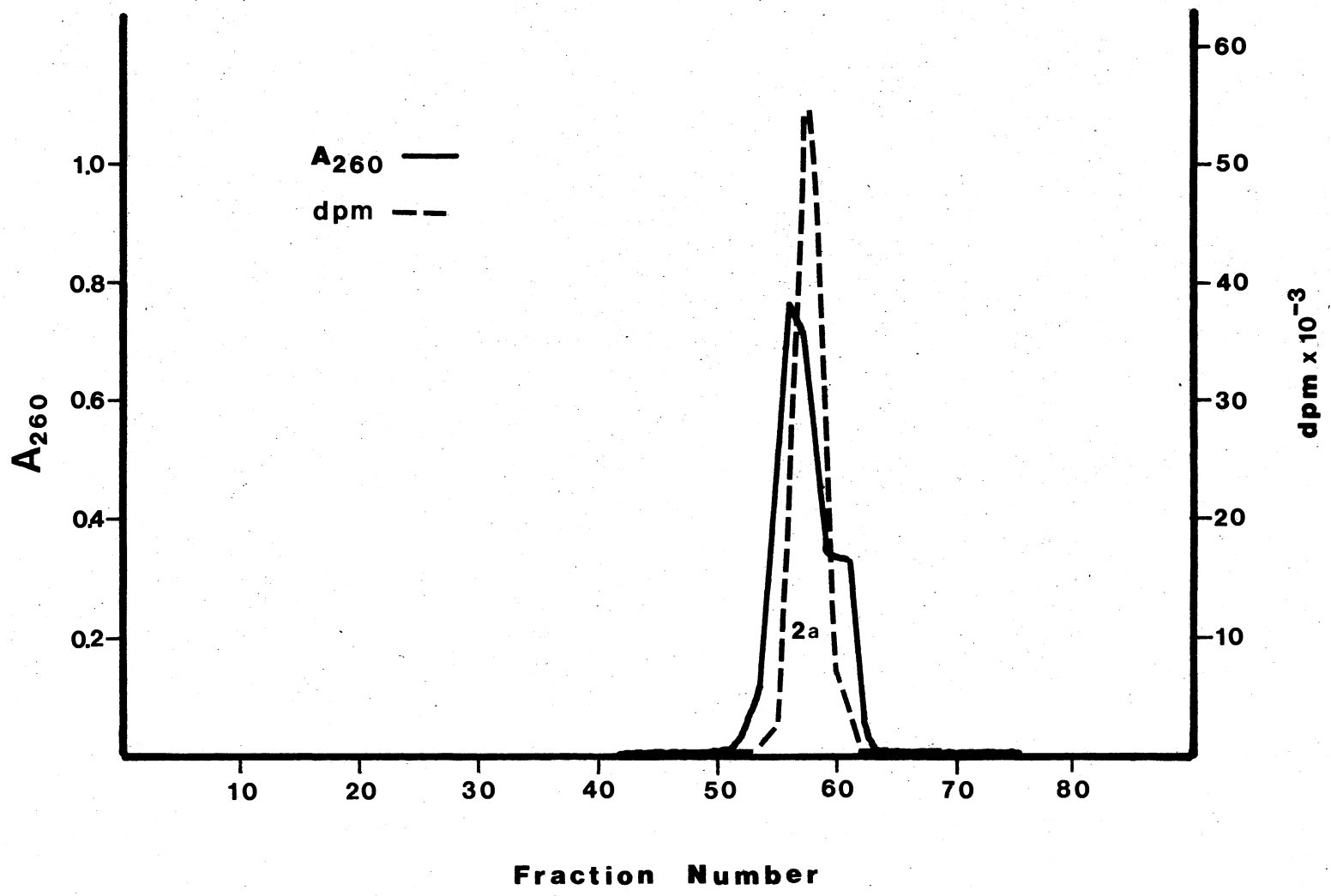


Figure 17. Sephadex G-10 Chromatogram of Fraction 2a

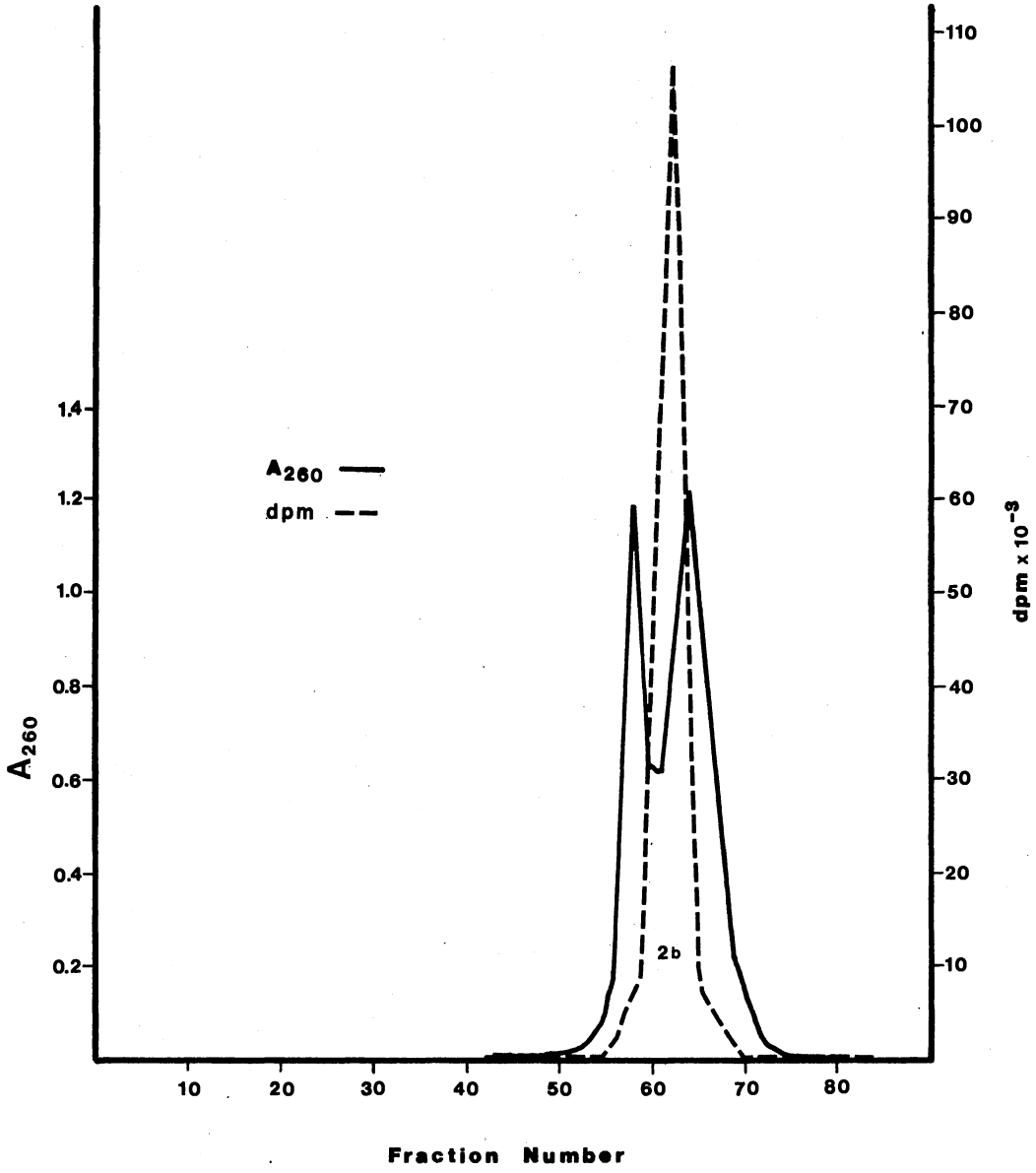


Figure 18. Sephadex G-10 Chromatogram of Fraction 2b

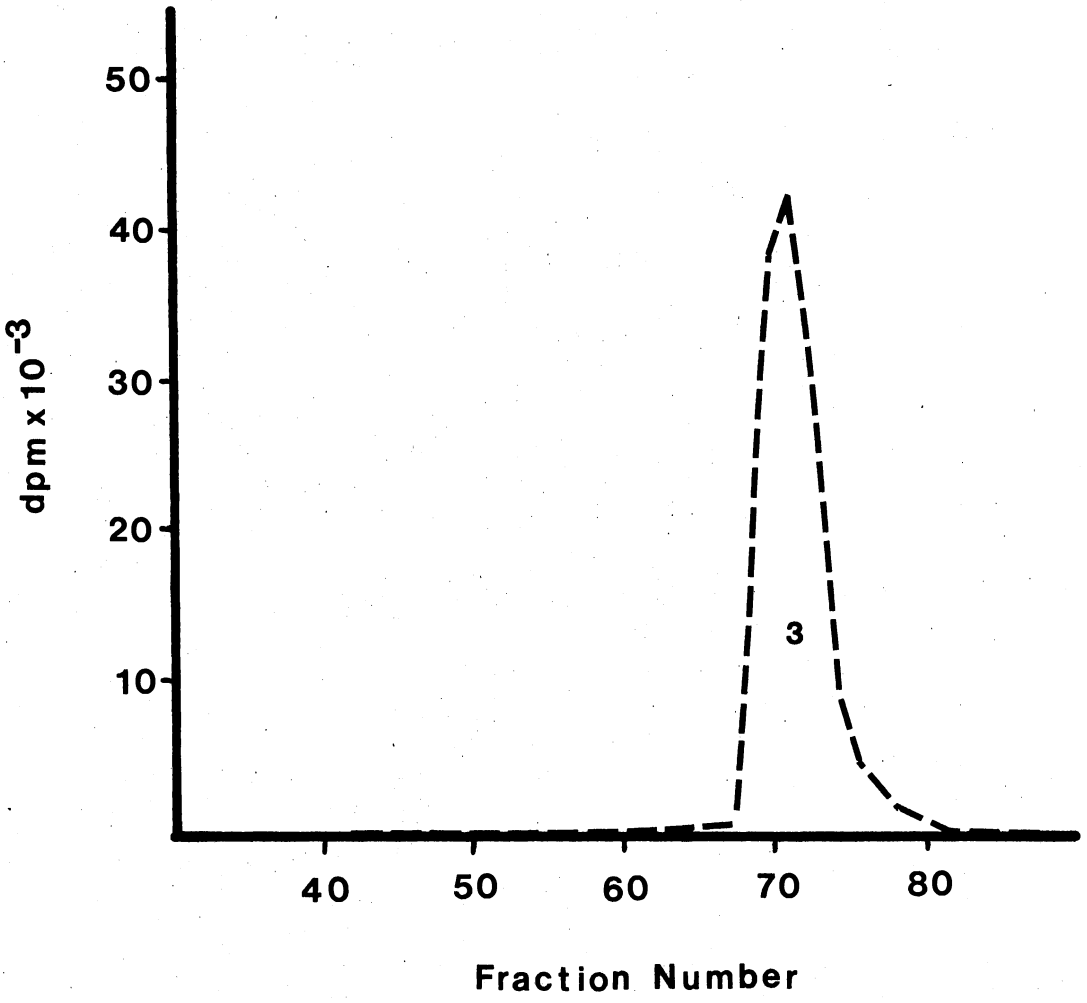


Figure 19. Sephadex G-10 Chromatogram of Fraction 3

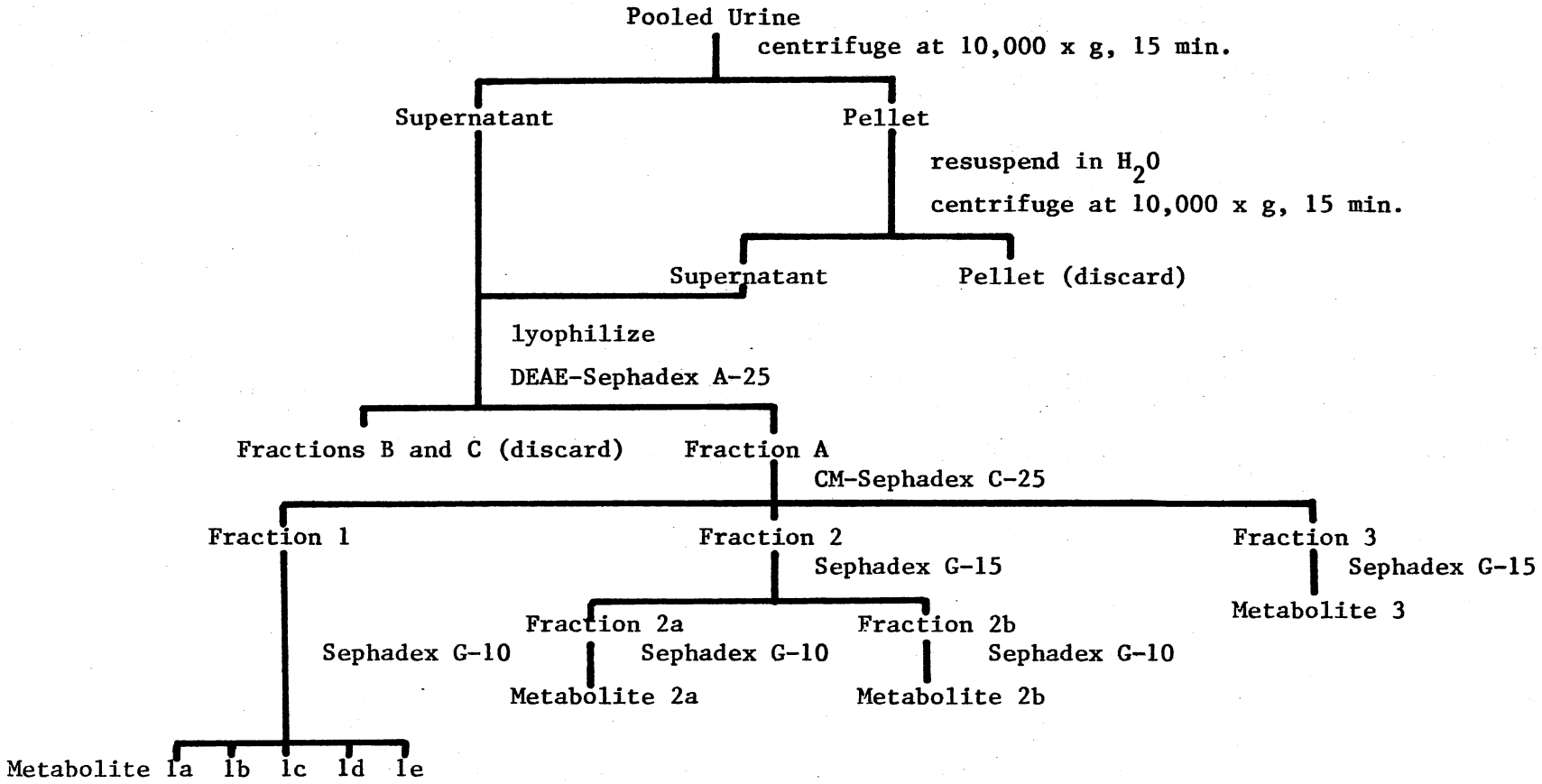


Figure 20. Isolation Scheme for Urinary Metabolites of 2,3-Dimethyl-5,6-Dihydropyrazine

TABLE I

URINARY METABOLITES OF MICE INJECTED WITH 2,3-^{14}C-2,3-DIMETHYL-5,6-DIHYDROPYRAZINE*

Metabolite	Percent Dose
1a	2.7
1b	1.7
1c	0.6
1d	3.8
1e	0.3
2a	8.2
2b	17.5
3	8.1
24 Hour Total	42.9

*Ten mice were administered doses of 267,000 dpm each

study and could be accomplished by incubation of the 2,3-dimethyl-5,6-dihydropyrazine with tissue and organ homogenates.

CHAPTER IV

SUMMARY

The synthesis and purification of 2,3-dimethyl-5,6-dihydropyrazine has been performed. The physical characteristics of the compound have also been established providing a basis for comparison for future studies concerned with the characterization of the metabolites of 2,3-dimethyl-5,6-dihydropyrazine.

Studies concerned with the biological activity of 2,3-dimethyl-5,6-dihydropyrazine in mice indicate a LD_{50} of 0.36 mg/g body weight. It was also shown that 43 percent of the administered dose is excreted in the urine in 24 hours.

Biochemical changes in the 2,3-dimethyl-5,6-dihydropyrazine do occur and at least five minor and three major metabolites have been indicated. Anion exchange, cation exchange, and molecular seive chromatographic techniques have been utilized in the purification of the urinary metabolites. No studies were conducted to define the nature of the major urinary metabolites due to time limitations.

Use of the techniques developed in this study might be helpful in future studies on tissue and organ homogenates as well as future studies on the urinary metabolites. Future studies should also be concerned with the comparison of the metabolites of 2,3-dimethylpyrazine with those of 2,3-dimethyl-5,6-dihydropyrazine.

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