METABOLISM OF NEPETALACTONE

IN FELIS DOMESTICA

By

GARY HOUSTON PRICE

Bachelor of Science

Central State College

Edmond, Oklahoma

1965

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1968

METABOLISM OF NEPETALACTONE

IN FELIS DOMESTICA

Thesis Approved:

George R. Waller
Thesis Adviser

George V. Odell

James E. Webster

D. Wickham
Dean of the Graduate College

ACKNOWLEDGMENTS

I wish to express my appreciation to my research adviser, Dr. George R. Waller, Associate Professor of Biochemistry at Oklahoma State University, for his guidance, counsel and encouragement during this research program.

I also am grateful to Drs. Fred E. Regnier and Hamid Auda for their suggestions and assistance, and to Dr. Earl D. Mitchell for his assistance in conducting mass spectrometry.

A special appreciation is due Dr. Bertis Glenn of the Veterinary Pathology Department for his help in administering doses and his advice on maintaining the experimental cats, and to Dr. Roger Panciera for the necropsies performed.

I wish to thank Drs. E. J. Eisenbraun and Karl Schorno for furnishing standard compounds and M. Wayne Adkins, glassblower, for his help in building special equipment.

I am indebted to the Biochemistry Department for financial support and use of its facilities.

My special thanks go to my wife, JoAnne, for her patience and encouragement during my course of study and her assistance in typing the thesis.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The widespread occurrence of monoterpenes in the plant kingdom and their effects on a largely herbivorous animal world presents an area of potential interest and importance to studies in biochemistry, toxicology, physiology, pharmacology, and related disciplines.

The response of the domestic cat, Felis domestica, to the monoterpenoid lactone, nepetalactone, led to an interest in its metabolic products and possible physiological and histological effects when ingested.

Of incidental interest is the fact that this same compound occurs in the diet of humans who use the crushed leaf of catnip, Nepeta cataria, as a flavoring agent in tea.

The observation that ingestion of nepetalactone by the cat failed to produce the response elicited by olfactory stimulation, followed by necropsy indicating the absence of permanent histological manifestations, caused the investigation to be concentrated on elucidation of the metabolites.

To achieve this end, a series of fractionation procedures was adopted, using both radioisotope and chromatographic methods. Identification of the primary metabolite was based on gas-liquid chromatographic retention times, R_f values on thin layer chromatography and the mass spectrum of a derivative.

CHAPTER II

LITERATURE REVIEW

The Metabolism of Monoterpenoids in Mammals

A pioneer investigator in the field of monoterpenoid metabolism was Hildebrandt, who found that Hildebrandt's acid (V) was produced by dogs and rabbits fed citral (VI) (1). Kuhn, et al., studied the degradation of dihydromyrcene, geraniol and geranic acid in rabbits and found that these compounds also underwent ω-oxidation to yield Hildebrandt's acid (2). Kuhn's primary interest was in the oxidation states of terminal methyl groups; much work was carried out in establishing that methyl groups were oxidized to carboxyl groups by way of the primary alcohol followed by the aldehyde. A review of this area, such as that by Sandermann (3), Williams (4) or, most recently, by Waller (5) indicates that almost always the C_{10} skeleton is retained, while the metabolism consists of oxidizing or reducing functional groups or double bonds, opening or closing rings, or conjugation with glucuronic acid or glycine prior to excretion, primarily in urine.

An example of hydroxylation of a methylene group of a monoterpene by a mammal is illustrated by the oxidation of camphor (VII) to 3-oxocamphor (VIII) or 5-oxocamphor (IX). Under similar conditions, dimethyl camphor (X) yielded the hydroxy compound (XI) in investigations carried out by Asahina and Ishidate (6). They also discovered that the methyl group in the beta position with respect to the carbonyl

group could also be oxidized; they were able to recover compounds in all three states of oxidation, alcohol (XII), aldehyde (XIII) and acid (XIV). Teresantol (XV) is formed by reduction of the carbonyl group of the alcohol (XVI). Cyclization of an acyclic monoterpenoid can also occur as illustrated by the formation of p-menthane-3,8-diol (XVII) from citronellal in the rabbit (7). The cyclization is probably due to the low pH of the gastric juice, and occurs in dilute HCl as well as *in vivo*. The diol is excreted as the glucuronide. Limonene (XIX), a plentiful substituent of citrus fruits, is oxidized by humans to uroterpenol (XX), which is excreted as the glucuronide (8). The conjugated diene, α-phellandrene (XXI), is oxidized to phellandric acid (XXII) which is partially excreted as the glycine conjugate, phellanduric acid (XXIII) by the sheep (9). Rabbits fed carvone (XXIV) excrete a glucuronide (7) and a dextrorotatory carbinol formed by reduction of the carbonyl group and saturation on one double bond (10). The glucuronides of menthol (XXVI) and pulegol (XXVII) are excreted by rabbits fed pulegone (XXVIII) (11). Menthone (XXIX) is reduced to d-neomenthol (XXX); it appears that a stereospecific enzyme may be involved, as the other possible reduction product, l-menthol, is not detected (12). Most of the carbonyl groups which occur within the rings of monocyclic terpenes apparently undergo reduction in mammals, in contrast to the previously cited oxidation of the terminal aldehyde group of citral to a carboxyl group. On the other hand, cyclocitral (XXXI) is reduced to cyclogeraniol (XXXII) in the rabbit and excreted as the glucuronide (13). Structures are shown in Fig. 1.

No information has been found concerning the fate of the methylcyclopentane nucleus or its bicyclic derivatives such as nepetalactone

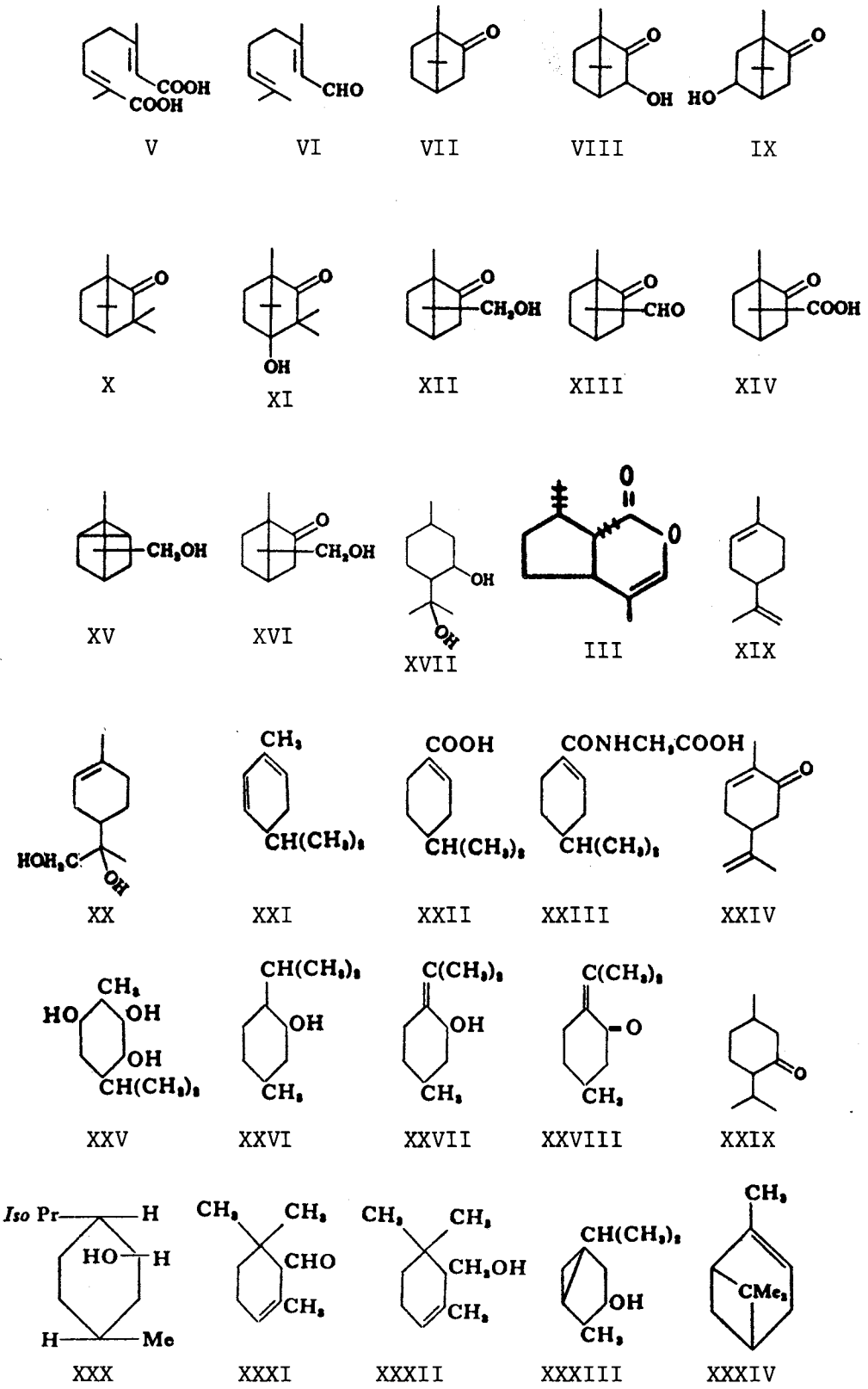


Figure 1. Some Monoterpenoid Compounds

in mammalian metabolism; however, the p-methyl isopropylcyclohexane carbon skeleton of most cyclic monoterpenes survives intact in form, changing only in oxidation state of functional groups, saturation or unsaturation of carbon-carbon bonds or conjugation with other types of compounds. One striking case of opening of a homocyclic ring occurs in the metabolism of the bicyclic terpene thujyl alcohol (XXXIII) by rabbits. A three-membered ring which probably has less structural stability than five or six-membered rings is opened in this case (14). α-pinene (XXXIV), a bicyclic compound with a four-membered ring, is converted by the rabbit to a glucuronide of a monocyclic alcohol, possibly p-menthanetriol (XXV) (15).

Nepetalactone

Nepetalactone (I) has been found to be the primary constituent of the essential oil of Nepeta cataria L., commonly known as catmint or catnip (16). Todd investigated the attraction and response of cats to N. cataria and to its essential oil which contains 80-95% nepetalactone (17). His hypothesis is that the response is due to a mimicing by nepetalactone of a pheromone, produced by tomcats and excreted in the urine, which brings about specific courtship behavior. This response to catnip has been shown to be hereditary and to occur in about half of sexually mature female cats tested.

Nepetalactone has also been found by Eisner (18) to be an insect repellant, as are some methylcyclopentane monoterpenoids produced by insects (19). It is proposed that the chief role of nepetalactone is protection of the plant against phytophagous insects, and that the response of the cat is only coincidental.

Nepetalactone undergoes delactonization in base to form alpha and delta nepetalic acid (II). Epinepetalactone (III), which differs from nepetalactone only at the 7a asymmetric carbon, yields the beta and gamma isomers of nepetalic acid on delactonization. Epinepetalactone also occurs as a constituent of catnip oil, in amounts ranging from about 1-20% (ratios of nepetalactone to epinetalactone range from 99.6:0.4 to 70:30). Oxidation of the aldehyde group of nepetalic acid yields the dibasic acid, nepetalinic acid (IV), of which there are four stereoisomers, alpha, beta, gamma and delta. Methylation by diazomethane yields dimethyl nepetalinate, which similarly occurs in four stereoisomers. The absolute stereochemistry and structure elucidation of this series and other methylcyclopentane monoterpenoids is based on the observations of McElvain, et al. (16, 20).

CHAPTER III

EXPERIMENTAL METHODS

Preparation of Nepetalactone-^{14}C

Biosynthesis of Nepetalactone-^{14}C. *Nepeta cataria* plants were placed in a plant growth chamber illuminated on two sides by Sylvania Gro-Lux fluorescent lamps. In the side arm of the chamber was placed 1 mc of $BA^{14}CO_3$ of specific activity 28 μc/mg (New England Nuclear Corp., Boston, Mass.). The system was closed and 5 ml of 1N sulfuric acid was injected into the side arm.

Purification of Nepetalactone-^{14}C from the Essential Oil. After 48 hours exposure, the excess $^{14}CO_2$ was evacuated from the chamber and trapped in 1N sodium hydroxide. The plants were removed, weighed, macerated and steam distilled for one hour. The steam distillate was saturated with sodium chloride and extracted three times with equal volumes of diethyl ether. The ether solution was dried overnight over anhydrous magnesium sulfate, filtered and concentrated under a nitrogen stream. The essential oil was chromatographed on Silica Gel G in hexane, acetone and ethanol (40:10:4 V/V). Purity was checked by gas-liquid chromatography. The column used was 10' x ¼" coiled glass, packed with 25% Apiezon L (Applied Science Labs, State College, Pa.) on Gas Pack S after silanizing by washing the column bore with dimethyldichlorosilane.

Administration of Nepetalactone

Nepetalactone in doses of 50-100 μl (42-84 mg) was placed in a small gelatin capsule which was then coated with mineral oil. The cat, under manual restraint, was force-fed the capsule..

Metabolism Chamber

The metabolism chamber used to collect urine, feces and expired carbon dioxide was fabricated from a 44 x 22 cm chromatography tank fitted with a perforated ¼" Lucite floor. The cover was formed from a 3 liter round bottom flask on which a lip was formed and fitted to the tank opening by means of a ground joint. A stopcock was placed under the outlet to collect urine. Two carbon dioxide traps were filled to 100 ml each with 1N sodium hydroxide and mounted in series with the air outlet. An aspirator on the distal trap maintained constant air flow through the system. All cold nepetalactone feeding experiments were carried out in a Hoeltge HB-33 Metabolism Cage (Hoeltge, Inc., Cincinnati, Ohio), dimensions 48 x 40 x 34 cm; fresh water and dry type food were supplied free-choice.

Gross Distribution of Radioactivity in Excreta

Collection of Excreta. Expired carbon dioxide was trapped in 1N sodium hydroxide as described above. Feces were steam distilled one hour and the pot residue lyophilized. Urine accumulated under the Lucite floor was drained from the stopcock as soon as possible after excretion and held frozen until analysis.

Isotope Analyses

Specific Activity of Nepetalactone-^{14}C. Purified nepetalactone-^{14}C was weighed on a Mettler Micro Balance. One ml of n-hexane was added and 10 μl of the solution was added to 15 ml of Bray's solution (21) and counted for ten minutes in a Packard Model 3314 Tri-Carb liquid scintillation counter. Benzoic acid-^{14}C (4.3 mg, 2.5 mc/mM, Research Specialties, Richmond, Calif.) was counted in 15 ml of Bray's solution to provide a measure of efficiency.

Radioactivity in Expired Carbon Dioxide. One ml of the combined sodium hydroxide trap solutions was counted in 15 ml of Bray's solution for ten minutes.

Radioactivity in Urine. One ml of each urine sample was counted ten minutes in 15 ml of Bray's solution.

Radioactivity in Feces. Fecal material (14.4 g) was collected and subjected to steam distillation for two hours. The distillate was saturated with sodium chloride and extracted with ethyl ether. After reducing the ether volume under nitrogen stream, the volume was adjusted to 5 ml and a .1 ml aliquot was counted in 15 ml of Bray's solution. The aqueous residue was frozen in an acetone-dry ice bath and lyophilized to dryness, yielding 5.9 g of dry material. Twenty mg of this material was counted by wet combustion, using the method of Van Slyke, et al. (22).

Distribution of Urine Radioactivity by pH Fractionation

Thirty ml of radioactive urine was saturated with sodium chloride and extracted with three volumes of ethyl ether. The aqueous fraction was acidified to pH 1 by addition of 1N hydrochloric acid, then

extracted again with three volumes of ethyl ether. The neutral and acid extracts were dried over anhydrous magnesium sulfate and the volumes reduced to 2 ml. Aliquots of .1 ml were counted in 15 ml of Bray's solution. A 1 ml aliquot of the acid aqueous urine fraction was similarly counted.

Preparation of Derivatives

Methylation of Acid Urine Extracts. Samples of the acid ether extracts of nepetalactone-fed and control urine were treated with an excess of diazomethane prepared by the method of Regnier, Eisenbraun and Waller (23). In a 500 ml flask, 150 ml of ether, 60 ml of Carbitol (diethylene glycol monoethyl ether), and a solution of 24 g of sodium hydroxide in 20 ml of water were cooled to 0°, followed by the addition of 7.1 g of EXR-101 (E. I. DuPont de Nemours & Company, Wilmington, Delaware). Magnetic stirring was begun and the mixture warmed slowly. At 15° to 20°C the evolution of diazomethane was noted. At 30° to 40°C the ether and diazomethane distilled and condensed to a bright yellow solution. On disappearance of yellow color in the reaction flask, the reaction was presumed complete. The receiving vessel was cooled with ice and ether added to the reaction flask when the volume became low. Yield was approximately 2 g diazomethane in ether solution.

To the acid ether extracts and to standard acids the ether solution of diazomethane was added dropwise until a permanent yellowish-green color (one to two minutes duration was observed and the evolution of nitrogen ceased). The excess solution was evaporated.

Trimethyl Silyl Esters. Bis-trimethylsilyl acetamide (BSA) which had been prepared by the method of Klebe, et al., (24) was added

dropwise to the nepetalactone-fed and control acid urine extracts and to standard acids. The mixtures were stirred and allowed to stand 15 minutes after which the excess BSA was evaporated off under nitrogen stream.

Gas-Liquid Chromatography

Column Specifications. Column 1 was the silanized Apiezon L glass column used for determination of purity of nepetalactone-^{14}C and previously described. Column 2 was a 10' x ¼" coiled glass column packed with 25% Carbowax 20M on acid-washed Chromosorb W.

Both columns were cured at 250°C.

Gas Chromatograph. A Barber-Colman Series 5000 gas chromatograph equipped with a hydrogen flame ionization detector and modified to permit interchange of columns with the mass spectrometer-gas chromatograph was used.

Analytical Conditions. Samples of acid-ether extracts of nepetalactone-fed and control urine, methylated acid-ether extracts of nepetalactone-fed and control urine, dimethyl nepetalinate, and α-nepetalinic acid were dissolved in anhydrous diethyl ether and chromatographed at a column temperature of 200°C, detector temperature of 250°C, injector temperature of 200°C, and a carrier gas flow rate of 60 cc He/min. at a pressure of 48 psi. For the flame detector, hydrogen was generated at a pressure of 12 psi and mixed with air at 38 psi. In all cases where direct comparison of retention times was made, the samples compared were run consecutively to minimize parameter variations. Injection volumes ranged from 0.2 to 5.0 μl.

Thin Layer Chromatography

Radioactivity Profile of Acid Fraction of Nepetalactone-^{14}C-Fed Urine. A sample of the acid-ether extract was spotted on a glass plate coated with 25.0 mg/cm^2 of Silica Gel G. The plate was developed in hexane:acetone:ethanol (40:10:4 V/V) and air-dried. The plate was divided into bands of 1 cm width parallel to the solvent front and each band was scraped from the plate and added to a vial containing 15 ml of Bray's solution. The vials were counted in a Packard Tri-Carb Liquid Scintillation Counter for periods of time such that 99% of the samples would be counted with an accuracy of ±1%.

Silicic Acid Column Chromatography of Acid-Ether Extract and α-Nepetalinic Acid

A silicic acid column was prepared according to the method of Marvel and Rands (25). The volume of the column was 18 cubic centimeters. Approximately 7.5 mg of α-nepetalinic acid was washed on the column in 1 ml chloroform and eluted with water-saturated chloroform. Fractions of 1 ml were collected and titrated to a phenol red end point with 0.0143N sodium hydroxide. Approximately 10 mg of acid-ether extracted urine solids was washed on the column in 1 ml of chloroform, eluted, collected and titrated as above. About 30 mg of the urine acid-ether extract was chromatographed on the column to remove as many impurities as possible, then was held for methylation, GLC analysis, and TLC analysis.

Trimethylsilyl Derivatives of α-Nepetalinic Acid and Acid-Ether Extract of Nepetalactone-Fed Urine. The trimethylsilyl derivatives of α-nepetalinic acid and the acid-ether extract were applied to a Silica

Gel G plate and developed in the hexane:acetone:ethanol system. After air-drying, the spots were detected by placing the plate in a closed glass tank filled with iodine vapor.

Free α-Nepetalinic Acid and Acid-Ether Extract. Samples of α-nepetalinic acid and the acid-ether extract dissolved in ether were applied to a Silica Gel G plate and developed in benzene:methanol:acetic acid (90:16:8 V/V) (26). After air-drying until no acetic acid could be detected by smell, the plate was sprayed with a solution of 0.04% bromcresol green in ethanol (27). The acid spots appeared yellow on a blue background.

Mass Spectrometry

Mass Spectrometer-Gas Chromatograph. The combination mass spectrometer-gas chromatograph used was the prototype LKB 9000 (Karolinska Institutet, Stockholm, Sweden) (28, 29).

Analytical Conditions. The mass spectrometry was carried out at an electron energy of 70 eV, ion source temperature 310°C, separator temperature 220°C, multiplier high voltage 1.9 kV, trap current 65 milliamps, and accelerator high voltage 3.5 kV. The gas chromatograph was operated at an oven temperature of 200°C, injection port temperature 200°C, and carrier gas flow 60 cc He/min.

The mass spectra were computer-plotted from tabular intensity data. A CalComp 565 plotter driven by an IBM 1620 computer using a Fortran II-D program was employed. Background spectra were taken prior to each sample and subtracted from the sample spectra. The mass spectra are reported in terms of relative intensity, the most abundant ion being taken as 100%.

CHAPTER IV

RESULTS AND DISCUSSION

Purity and Specific Activity of Nepetalactone-^{14}C

Gas-liquid chromatographic (GLC) analysis of the purified nepetalactone-^{14}C showed two peaks (Fig. 2), corresponding to approximately 99% and 1% of the mixture tested. Comparison of the retention times of the 1% peak and epinepetalactone (III), which proved to separate nicely from nepetalactone under the conditions employed, indicated that epinepetalactone was the minor component. Further evidence for the identification of the minor peak was the fact that the R_f value of epinepetalactone in the thin layer chromatography system used in preparation was nearly the same as that of nepetalactone; furthermore, the nepetalactone:epinepetalactone ratio obtained from analysis of other Nepeta cataria plants had been found by Regnier, et. al., (23) to be 99.6:0.4.

N. cataria plants weighing 115 g yielded 116.4 mg of nepetalactone-^{14}C, possessing a specific activity of 1458 dpm/mg. This per cent yield was in agreement with that obtained in other experiments, which normally ranged from 0.1-0.15%.

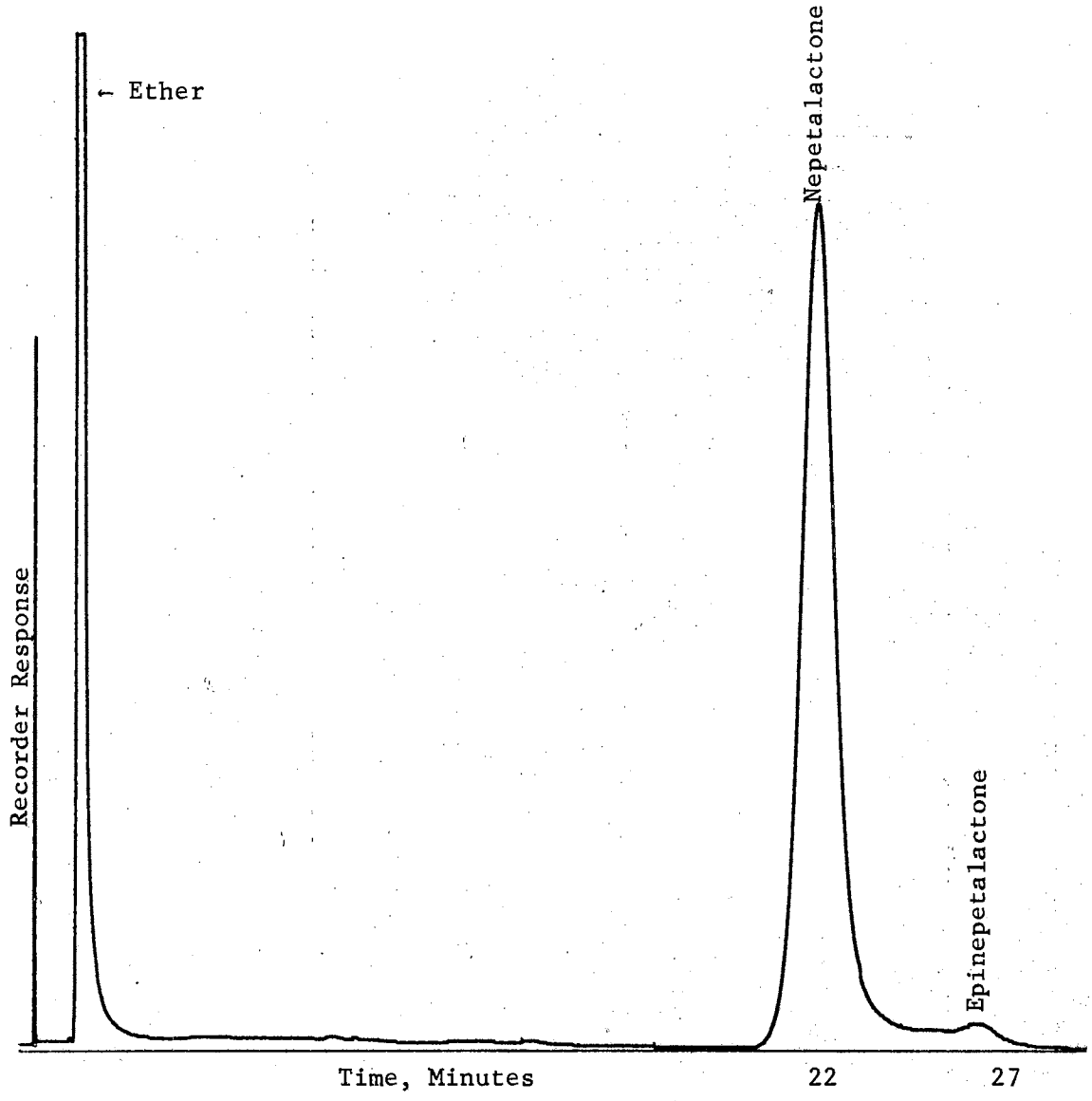


Figure 2. Gas-Liquid Chromatographic Analysis of Nepetalactone-^{14}C - Determination of Purity

Gross Distribution (Radioactivity) from Excreta

Of a total administered dose of 61,250 dpm (42.0 mg of purified nepetalactone-^{14}C), 86.0% was recovered from urine, 12.1% from expired $^{14}CO_2$, and 1.6% from steam volatile compounds in feces. The non-steam volatile residue from feces yielded no measurable radioactivity. The low recovery from the feces indicated virtually quantitative absorption of nepetalactone. A significant amount is probably degraded by at least one decarboxylation as indicated by the release of $^{14}CO_2$, although radioactive compounds possessing fewer than ten carbon atoms have not as yet been recovered from the urine.

Only the first urine sample collected in any experiment contained substantial levels of radioactivity, about twenty times as much as the second sample. Ninety-six hours following administration of nepetalactone-^{14}C it was observed that no further ^{14}C was eliminated in the urine.

Distribution of Radioactivity in Acid and Neutral Ether Extracts of Urine

Approximately 20% of the total radioactivity present in the urine was ether extractable at pH 6.2, the normal pH value of several cat urine samples tested. After acidification to pH 1, another 75% was ether extractable. The 5% remaining in the aqueous phase at pH 1 was not studied further.

Gas-Liquid Chromatographic Analyses of Acid-Ether Extracts

GLC analysis of the pH 1.0 acid-ether extract of nepetalactone-fed urine (Fig. 3A) shows a large peak (Unknown I) at 60 minutes retention time which does not occur in the corresponding analysis of control urine (Fig. 3B). The Unknown I peak disappears on methylation with diazomethane, and a new peak, Unknown II, is observed (Fig. 3C). The marked change in retention time of the predominant peak after methylation provided the first positive evidence that the metabolite was a carboxylic acid.

GLC Analyses of Dimethyl Nepetalinate and Methylated Acid-Ether Extracts of Nepetalactone-Fed and Control Urine

The retention times of dimethyl nepetalinate (Fig. 4B) and Unknown II (Fig. 4A) were identical when either the Carbowax or Apiezon column was used. A mixture (co-chromatography) of the methylated acid-ether extract and dimethyl nepetalinate was injected under identical conditions and a more intense symmetrical sharp peak (Fig. 4C) was observed. No significant peak with the same retention time was observed in the analysis of a methylated acid-ether extract of control urine (Fig. 4D). These data present strong evidence that the metabolite is methylated by diazomethane to yield dimethyl nepetalinate. A logical precursor of the dimethyl nepetalinate is one of the nepetalinic acid isomers (20); therefore, experiments were devised to determine if a free nepetalinic acid were present in the urine.

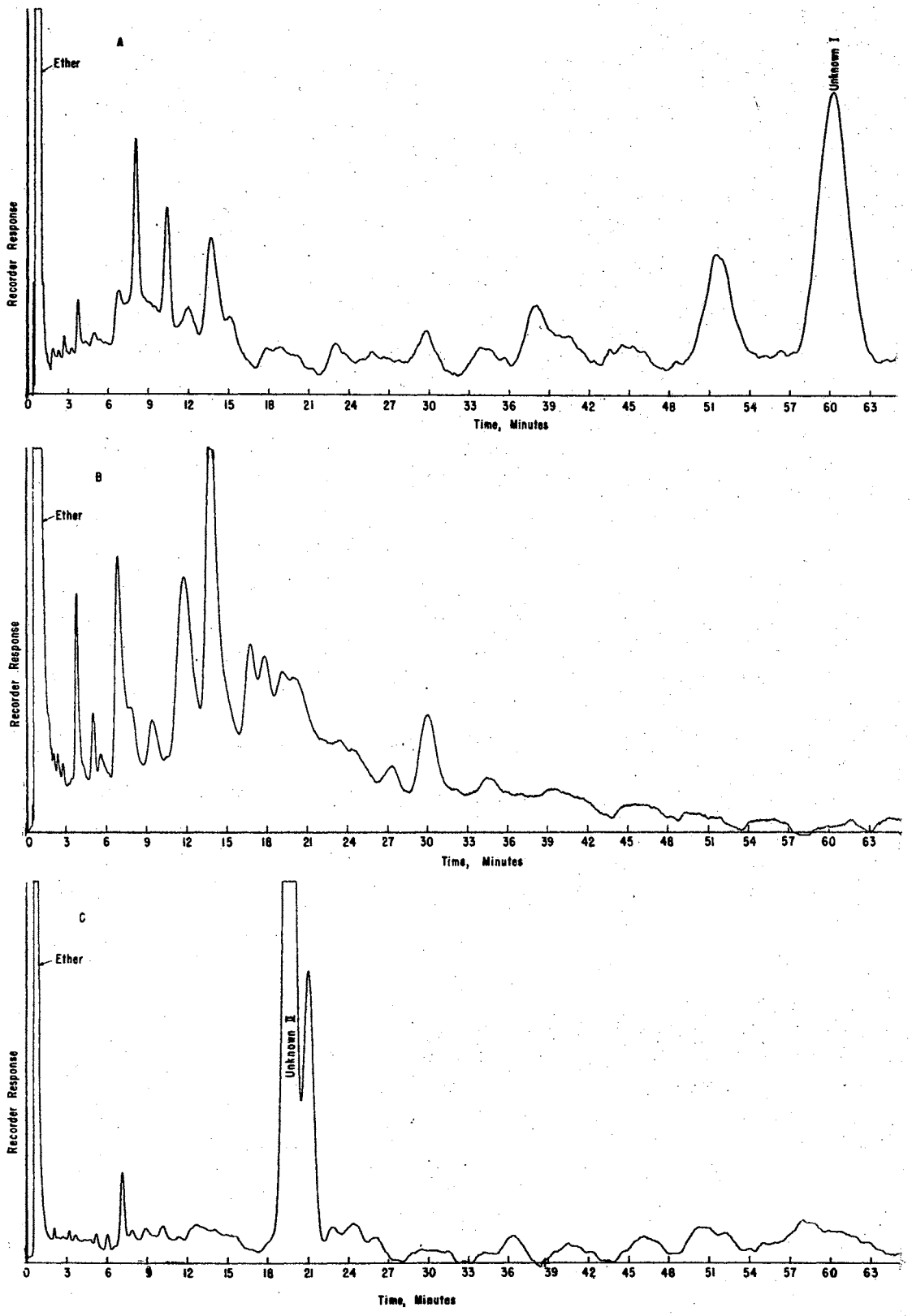


Figure 3. GLC Analysis of Acid-Ether Extracts. A - Nepetalactone-Fed. B - Control. C - Methylated Nepetalactone-Fed.

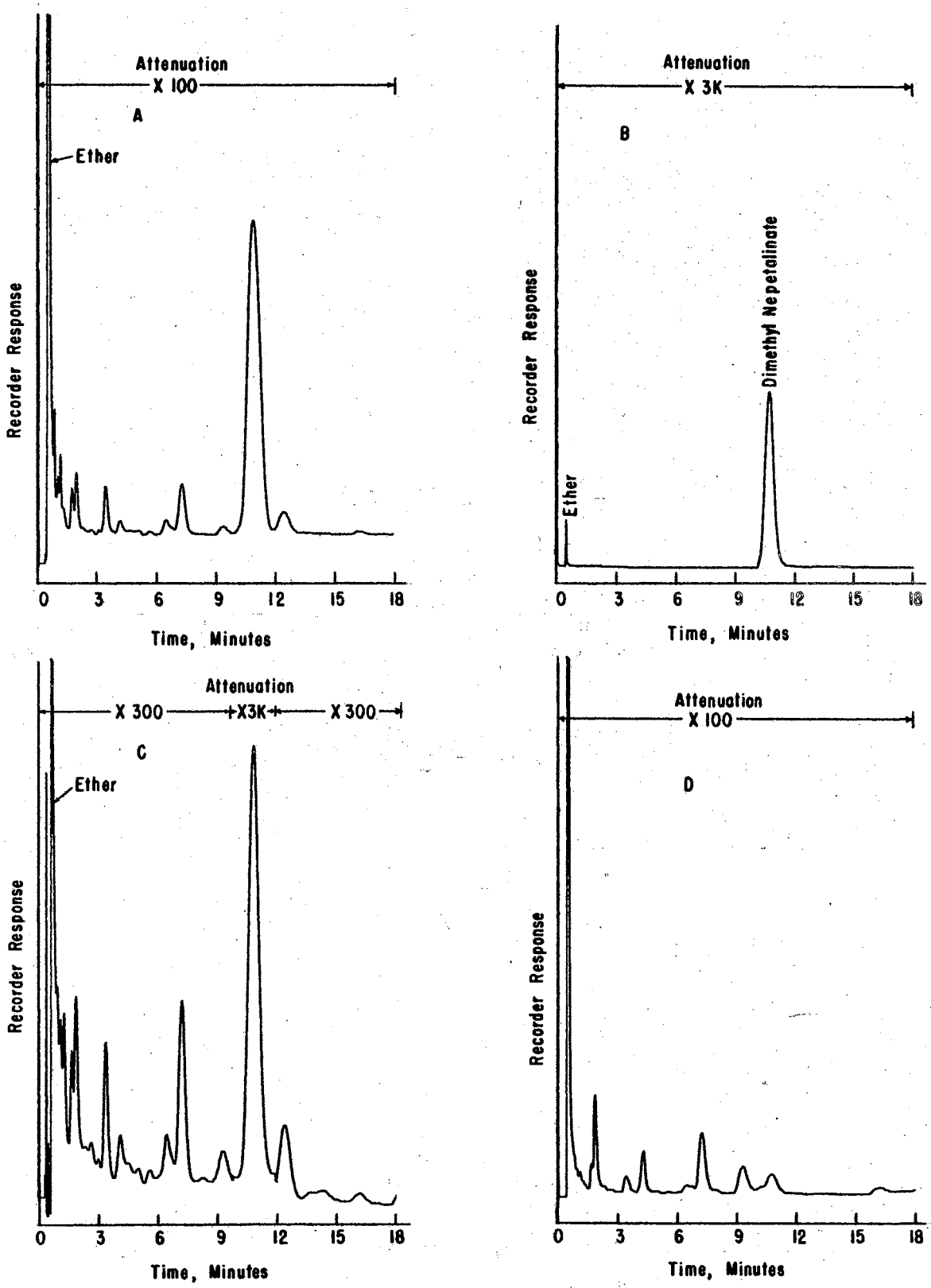


Figure 4. GLC Analyses of Dimethyl Nepetalinate and Methylated Acid-Ether Extracts. A - Methylated Nepetalactone-Fed Urine. B - Dimethyl Nepetalinate. C - Dimethyl Nepetalinate and Methylated Nepetalactone-Fed. D - Control.

GLC Analyses of α-Nepetalinic Acid and Acid-Ether Extract of Urine

The predominant peak observed in GLC analysis of the acid-ether extract on the Apiezon L column (Fig. 5A) showed a retention time identical to that of α-nepetalinic acid (Fig. 5B). Since α-nepetalinic acid readily forms the anhydride at 180°C (31), it is almost certain that it is this form which is observed in GLC analysis.

Thin Layer Chromatography of α-Nepetalinic Acid and Acid-Ether Extracts of Nepetalactone-Fed Urine

TLC of the acid-ether extract and α-nepetalinic acid in the benzene:methanol:acetic acid system (Fig. 6B) shows that one spot from the extract corresponds in R_f to α-nepetalinic acid.

TLC of the trimethylsilyl derivatives of α-nepetalinic acid and the acid-ether extract in the hexane:acetone:ethanol system (Fig. 6A) show identical R_f values for the TMS-nepetalinate derivative and one of the compounds in the TMS-derivatized acid-ether extract.

Radioactivity Profile of the Acid-Ether Extract after TLC

TLC of the acid-ether extract from nepetalactone-^{14}C-fed urine shows a spot corresponding in R_f value to α-nepetalinic acid (Figs. 7A, 7B). The radioactivity profile shows that the highest region of activity corresponds also to the R_f value of α-nepetalinic acid (Fig. 7C).

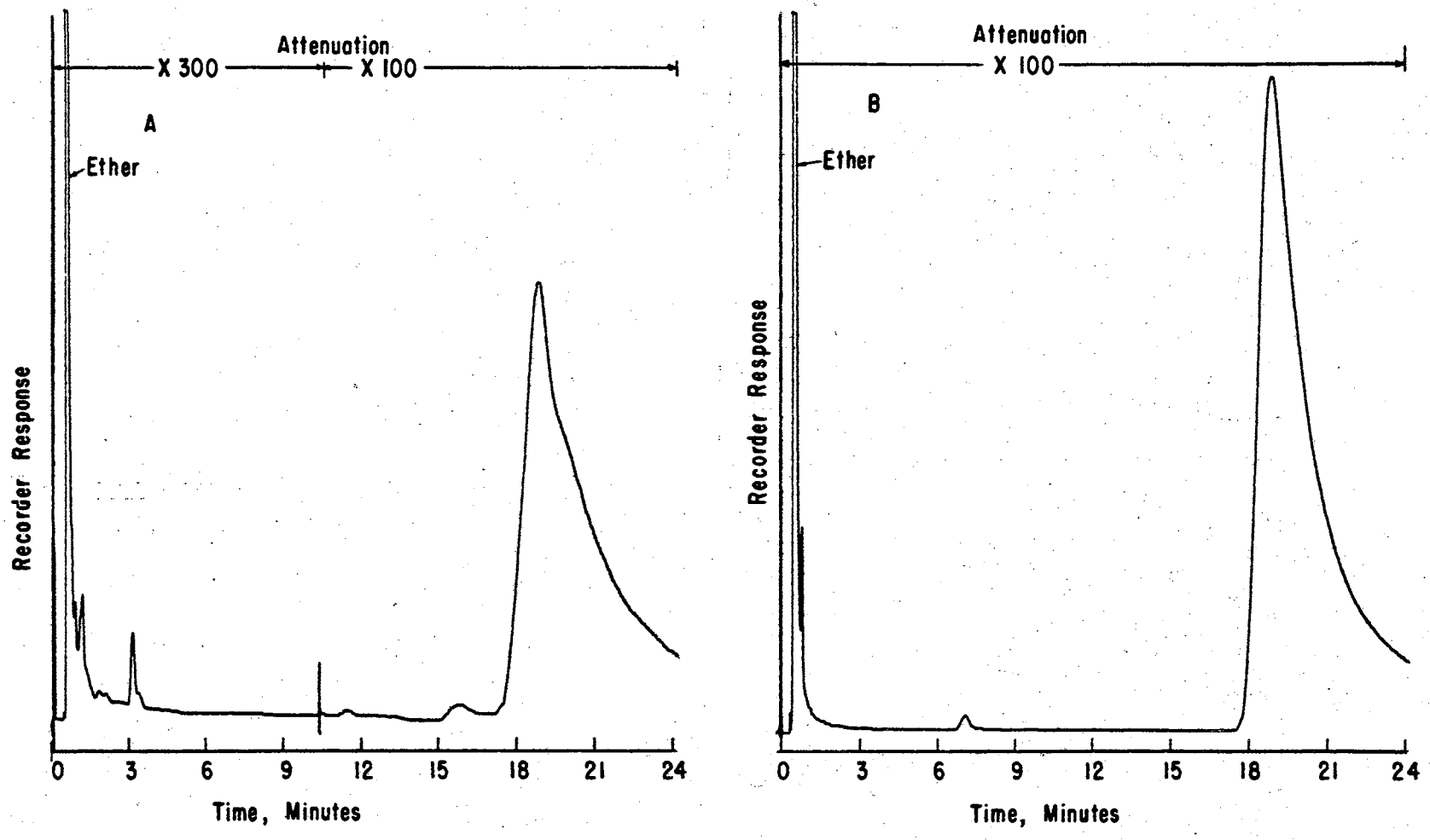


Figure 5. GLC Analyses of α-Nepetalinic Acid and Acid-Ether Extract of Nepetalactone-Fed Urine. A - Acid-Ether Extract. B - α-Nepetalinic Acid.

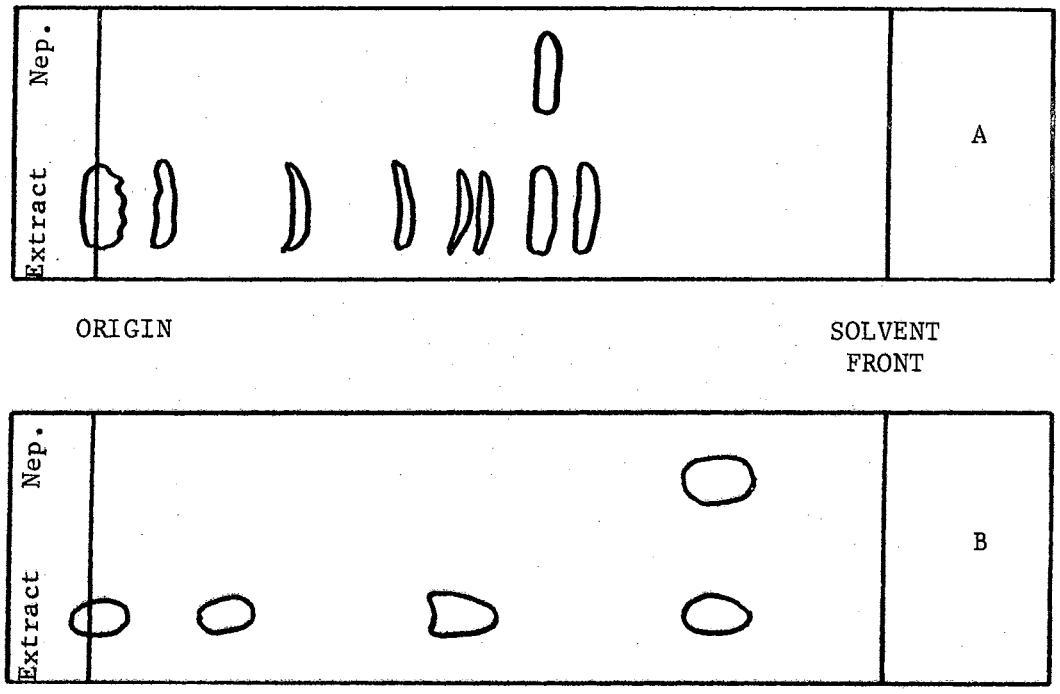


Figure 6. TLC Analyses of Acid-Ether Extract of Nepetalactone-Fed Urine and α-Nepetalinic Acid. A - Hexane:Acetone:Ethanol Solvent System, All Compounds Treated with Bis-trimethylsilyl acetamide. B - Benzene:Methanol:Acetic Acid Solvent System.

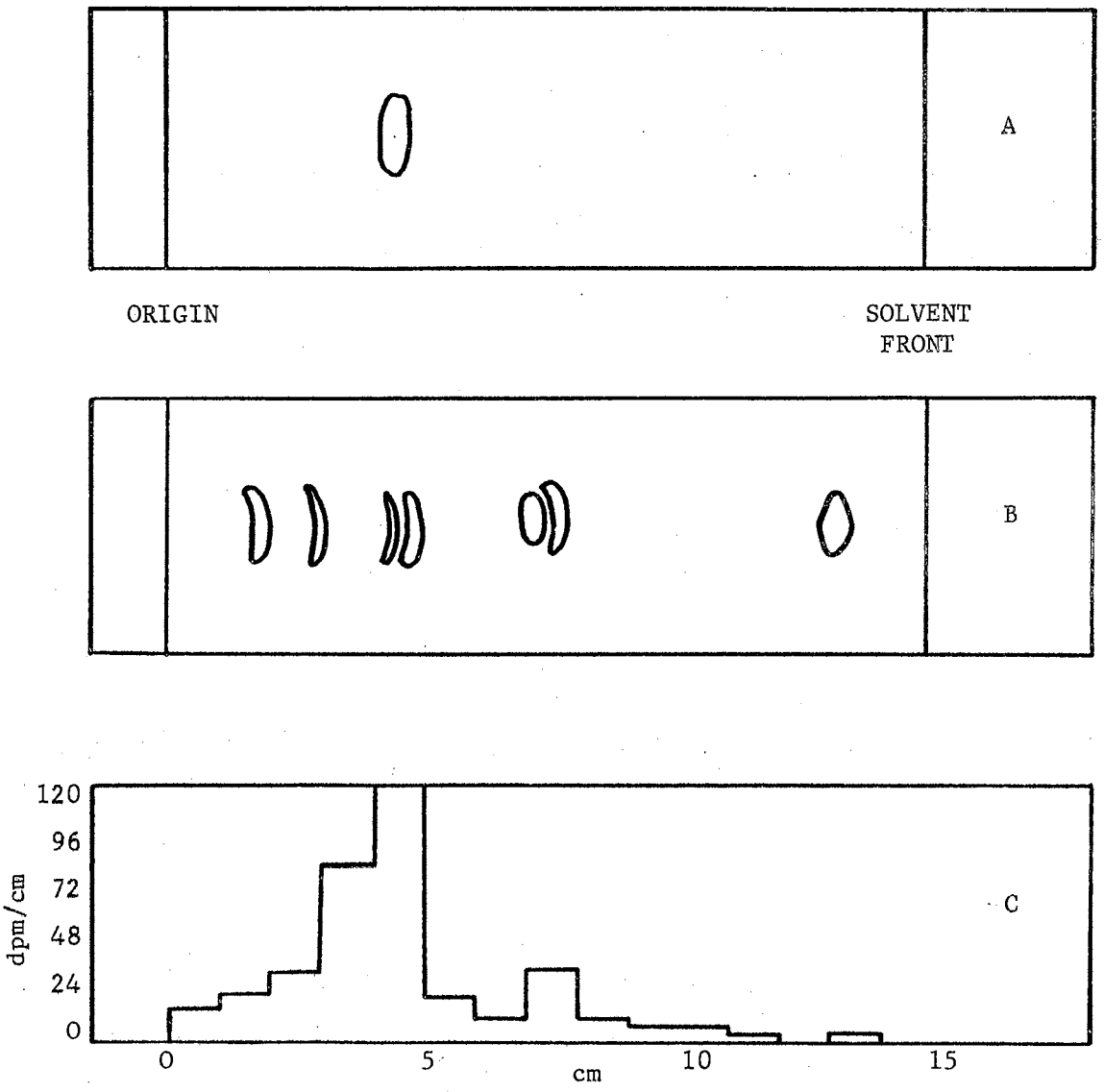


Figure 7. TLC Analysis of Acid-Ether Extract of Nepetalactone-^{14}C Fed Urine and α-Nepetalinic Acid. A - Nepetalinic Acid. B - Acid-Ether Extract. C - Radioactivity Profile, dpm/cm vs. cm.

Silicic Acid Chromatography of α-Nepetalinic Acid and Acid-Ether Extract

Upon chromatography both α-nepetalinic acid and the acid-ether extract showed an acidic compound with an elution volume 9 ml (Fig. 8). A sample of the urine acid collected from 8 to 10 ml elution volume was run on the benzene:methanol:acetic acid TLC system concurrently with α-nepetalinic acid, and the two acid spots revealed by bromcresol green spray had identical R_f values.

About 30 mg of the urine solids were chromatographed on the silicic acid column in three batches; the 8-10 ml fractions were combined and chromatographed on the benzene:methanol:acetic acid TLC system. The acidic band with the same R_f as α-nepetalinic acid was then eluted, treated with diazomethane, and injected on the gas chromatograph (Apiezon L column, same conditions as previously described), followed by the injection of a dimethyl nepetalinate standard. The methylated urine acid and the dimethyl nepetalinate had identical retention times.

Mass Spectrometry of Dimethyl Nepetalinate and Unknown II

Comparison of the mass spectra of dimethyl nepetalinate and Unknown II (Figs. 9A, 9B) provides further evidence that dimethyl nepetalinate occurs in the urine after treatment with diazomethane. The ten most abundant peaks in both spectra are m/e 81, 168, 109, 141, 197, 88, 59, 41, 67 and 153, with very minor variation. Both spectra show a molecular ion, $M^+ = 228$.

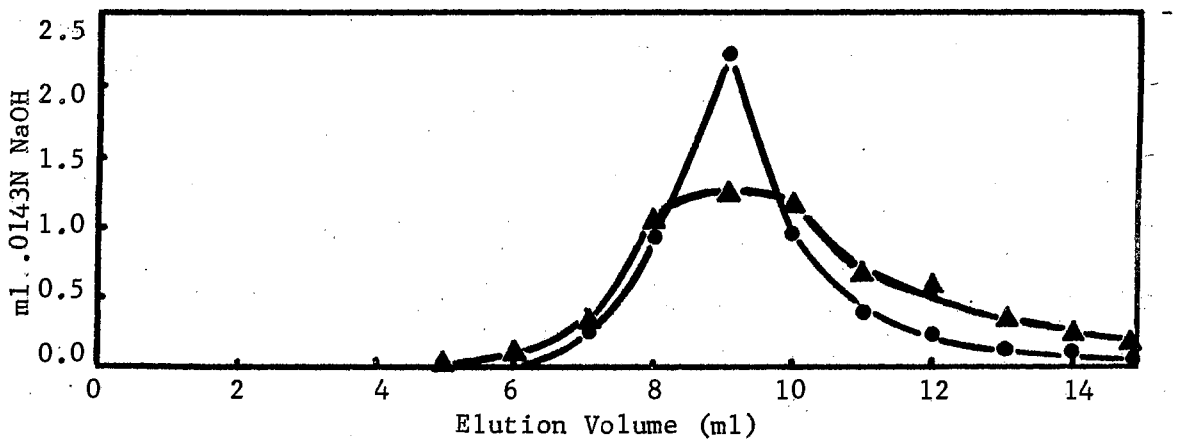


Figure 8. Silicic Acid Chromatography of Acid-Ether Extract of Nepetalactone-Fed Urine and α-Nepetalinic Acid. Circles Represent α-Nepetalinic Acid, Triangles Represent the Acid-Ether Extract.

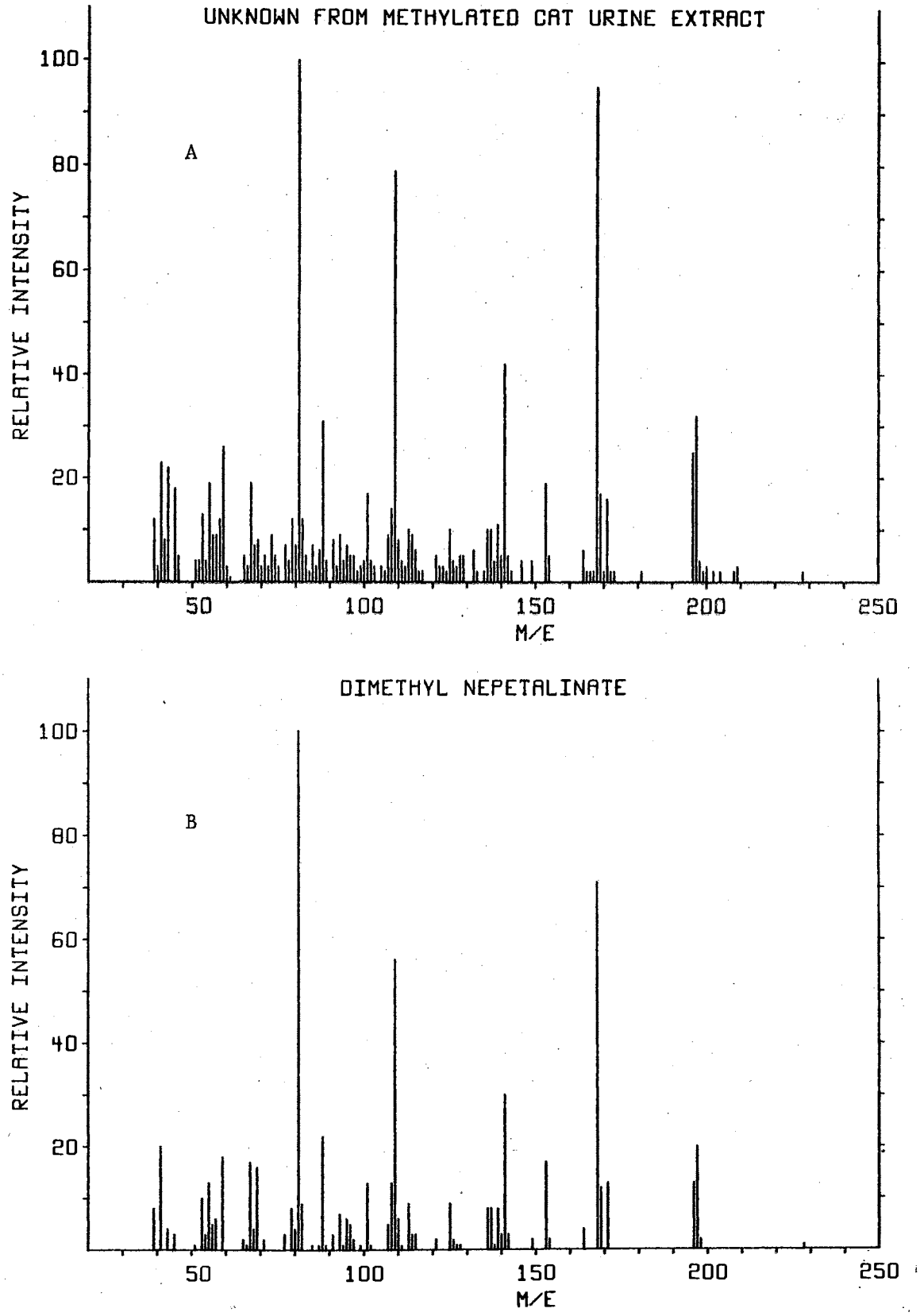
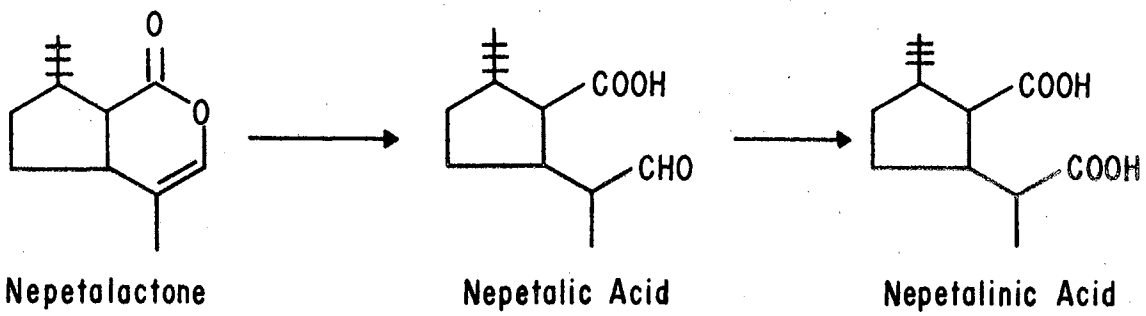


Figure 9. Mass Spectra of Dimethyl Nepetalinate and Unknown II. A - Unknown II. B - Dimethyl Nepetalinate.

Probable Pathway of Metabolism of Nepetalactone in *Felis domestica*

Analysis of the urine of a domestic cat which had been orally administered the monoterpenoid lactone, nepetalactone, demonstrated the presence of a nepetalinic acid in substantial quantities. Two steps are probably involved; first, an opening of the lactone ring to form nepetalic acid and, second, oxidation of the aldehyde group to a carboxyl group (Fig. 10). Opening of the lactone ring may be only a chemical reaction brought about by basic conditions of the small intestine, in which case equal amounts of the alpha and delta isomers of nepetalic acid would be formed. If a lactonase, such as that involved in the delactonization of an intermediate in camphor oxidation by a pseudomonad (32), were involved in opening the ring, stereospecificity should be observed; either the alpha or delta isomer should predominate. The ratio of alpha:delta would be expected to carry through the subsequent oxidation and methylation steps. The alpha and delta dimethyl nepetalinates are distinguishable by use of a capillary column on the gas chromatograph (30).

In keeping with the observation that some relationship between the catnip response and sexual attraction does exist (17), young sexually mature female cats (two) were used in the feeding experiments. Two experiments were conducted with isotopically labeled material, and ten experiments with unlabeled nepetalactone. Since no response was noted when the nepetalactone was orally administered, it does not seem reasonable to assume that the age or sex of the animal has any direct bearing on the metabolism of nepetalactone.



Nepetalactone I

Nepetalic Acid II

Nepetalinic Acid IV

Figure 10. Probable Pathway of Metabolism of Nepetalactone in F. domestica.

CHAPTER V

SUMMARY

Nepetalactone in doses of 42-84 mg may be orally administered to cats with no marked physiological or histological effects. The primary metabolite recovered from urine has been found by means of thin layer, gas-liquid, and silicic acid chromatography, as well as mass spectrometry, to be α-nepetalinic acid. Twenty-four to thirty hours after administration of nepetalactone-^{14}C, more than eighty per cent of the administered radioactivity may be recovered from urine, the remainder being respired as $^{14}CO_2$ with only a small amount in the feces. Urinary excretion of radioactivity is insignificant after about ninety-six hours.

A SELECTED BIBLIOGRAPHY

(1) Hildebrandt, H., Arch. Exp. Path. Pharmak. 45, 110 (1901).

(2) Kühn, R., Köhler, F., and Köhler, L., Hoppe-Seyl. Z. Physiol. Chem. 242, 171 (1936).

(3) Sandermann, W., in "Comparative Biochemistry," III, Florkin and and Mason (eds.), Academic Press (1962).

(4) Williams, R. T., "Detoxification Mechanisms," J. Wiley and Sons, N. Y. (1959), pp. 519-544.

(5) Waller, G. R., in "Recent Progress in the Chemistry of Fats and other Lipids," V. 10, R. T. Holman (ed.) Pergamon Press, London, in press.

(6) Asahina, Y., and Ishidate, M., Ber. 66, 1673 (1933); 67, 71 (1934); 68, 947 (1935).

(7) Fischer, F. G., and Bielig, H. J., Hoppe-Seyl. Z. Physiol. Chem. 266, 73 (1940).

(8) Wode, A. P., et al., Biochem. J. 101, 727 (1966).

(9) Wright, S. E., University of Queensland Papers 1, 25 (1945).

(10) Hildebrandt, H., Hoppe-Seyl. Z. 36, 141, 457 (1902).

(11) Teppati, R., Arch. int. Pharmacodyn. 57 (1937).

(12) Williams, R. T., Biochem. J. 34, 690 (1940).

(13) Hildebrandt, H., Beitr. chem. Physiol. Path. 4, 251 (1904).

(14) Hämäläinen, J., Skand. Arch. Physiol. 27, 141 (1921).

(15) Hämäläinen, J., Biochem. Z. 41, 241 (1912).

(16) McElvain, S. M., Bright, R. D., and Johnson, P. R., J. Am. Chem. Soc. 63, 1558 (1941).

(17) Todd, N. B., "The Captnip Response," unpublished Ph.D. Thesis, Harvard University, Cambridge, Massachusetts.

(18) Eisner, T., Science 148, 1218 (1965).

(19) Fraenkel, G. S., Science 129, 1466 (1959).

(20) McElvain, S. M., et al., J. Am. Chem. Soc. 77, 1588, 3383 (1955).

(21) Bray, G. A., Anal. Biochem. 1, 279 (1960).

(22) Van Slyke, D. D., et al., J. Biol. Chem. 191, 299 (1951).

(23) Regnier, F. E., Eisenbraun, E. J., and Waller, G. R., Phytochemistry 7 (1967), in press.

(24) Klebe, J. F., Finkbeiner, H., and White, D. M., J. Am. Chem. Soc. 88, 3390 (1966).

(25) Marvel, C. S., and Rands, R. D., Jr., J. Am. Chem. Soc. 72, 2642 (1950).

(26) Petrowitz, H. J., and Pastuska, G., J. Chromatogr. 7, 128 (1962).

(27) Braun, D., and Geenen, H., J. Chromatogr. 7, 56 (1962).

(28) Ryhage, R., Arkiv. Kem. 26, 305 (1967).

(29) Waller, G. R., Proc. Okla. Acad. Sci. 47, in press.

(30) Regnier, F. E., Ph.D. Thesis, Oklahoma State University, Stillwater, Oklahoma.

(31) Eisenbraun, E. J., personal communication.

(32) Conrad, H. E., et al., Tetrahedron Letters 10, 561 (1965).

VITA

Gary Houston Price

Candidate for the Degree of

Master of Science

Thesis: METABOLISM OF NEPETALACTONE IN FELIS DOMESTICA

Major Field: Chemistry (Biochemistry)

Biographical:

Personal Data: Born in Oklahoma City, Oklahoma, January 30, 1940, the son of Boone D. and Kathryn S. Price.

Education: Attended grade school in Arcadia, Oklahoma City, and Edmond, Oklahoma; graduated from Oklahoma City McGuinness High School in 1958; attended Oklahoma City University and St. Gregory's College, Shawnee, Oklahoma; received the Bachelor of Science degree from Central State College, Edmond, Oklahoma, with majors in biology and chemistry, in June, 1965; completed requirements for the Master of Science degree in July, 1967.

Professional Experience: United States Army Reserve, 1957-1967; Electronics Technician, Ampex (Computer Products Division), Culver City, California, 1962; NDEA Graduate Research Fellow at Oklahoma State University, 1965-1967.

Professional and Honorary Societies: Phi Lambda Upsilon