Feline Attractant, cis,trans-Nepetalactone: Metabolism in the Domestic Cat

Abstract. cis,trans-*Nepetalactone, the biologically active component of catnip, was force-fed to the domestic cat. When the* ^{14}C*-labeled compound was fed, 86 to 94 percent of the radioactivity was recovered in the urine, 1 to 2 percent was found in the feces, and 1 to 12 percent was collected as carbon dioxide. The major (50 to 75 percent) metabolite was α-nepetalinic acid, which was excreted in the urine together with small amounts of dihydronepetalactone, unchanged* cis,-trans-*nepetalactone, and several unidentified compounds. No marked physiological or histological effects were observed when 20 to 80 milligrams of* cis,-trans-*nepetalactone was administered orally. This is the first report on the metabolism of a mammalian attractant.*

The olfactory response of the domestic cat *Felis domestica* to the methylcyclopentane monoterpenoid *cis-trans*-nepetalactone (*1*, *2*), which constitutes 70 to 99 percent of the essential oil of the catnip plant *Nepta cataria*, led us to study its metabolism and possible physiological and histological effects when ingested. The capacity for the cat to give the response is inherited as an autosomal dominant. The response has been characterized as (i) sniffing, (ii) licking, and chewing with head shaking, (iii) chin and cheek rubbing, and (iv) headover roll and body rubbing; however, none of these automatisms was unique to nepetalactone, since each of them can also be associated with normal sexual or ingestive behavior (*2*). We now show that orally administered *cis,trans*-nepetalactone to the domestic cat results in the excretion of α-nepetalinic acid (2-carboxy-3,α-dimethylcyclopentaneacetic acid) (50 to 75 percent) in the urine and that no marked physiological or histological effects were observed.

cis,trans-Nepetalactone-^{14}C was photosynthetically prepared by administering $^{14}CO_2$ to selected *Nepeta cataria* L. plants (*3*). Preparative thin-layer and gas-liquid chromatography (*4*) of the steam distillate yielded pure compound which was used in the metabolism studies. The cat, under manual restraint, was force-fed a gelatin capsule containing either the ^{14}C-labeled (three experiments) or the unlabeled (14 experiments) *cis,trans*-nepetalactone (20 to 80 mg). Cats were starved for 24 hours before the capsule was administered; however, their hunger condition apparently had no qualitative effect on the metabolism of *cis,trans*-nepetalactone. In the experiment with labeled material, the cat was placed in an enclosed glass metabolism chamber, and the urine, feces, and expired CO_2 were collected; for experiments with unlabeled material a conventional metabolism cage was used. The cats represented three different parental lines. Three young, sexually mature females, one sexually mature male, and one sexually immature male were used. The urine was sequentially extracted with diethyl ether at *p*H 7 and *p*H 2, the yield being 80 to 90 percent of the total radioactivity.

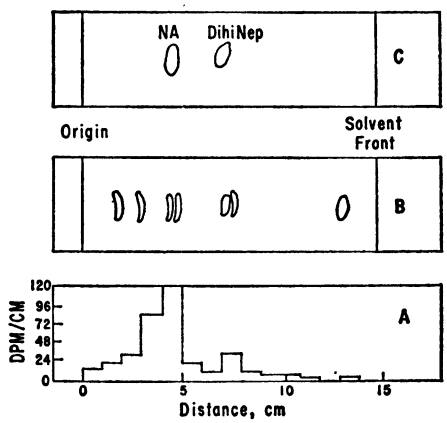

Fig. 1. Thin-layer chromatography tracing of ether extract of urine from a cat fed *cis,trans*-nepetalactone-^{14}C. (A) Radioactivity profile; (B) ether extract; (C) standard α-nepetalinic acid (*NA*) and dihydronepetalactone (*DihiNep*).

Since the results were qualitatively the same with all animals, with no outward manifestation of any physiological response being observed when *cis,trans*-nepetalactone was orally administered, it was concluded that the age or sex of the animal apparently has no direct bearing on the metabolism of nepetalactone. Histologic examination of tissue at autopsy indicated the absence of permanent alteration or damage.

Most of the radioactivity was excreted in the urine (86 to 94 percent) with a lesser amount recovered as expired $^{14}CO_2$ (1 to 12 percent) (*5*) and with a small amount in the feces (maximum of 2 percent) (*6*). Approximately 75 percent of the radioactivity was excreted during the first 24 hours, and excretion was complete after 96 hours. In one experiment, an animal was killed after 5 hours in the metabolism chamber, and about 60 percent of the ^{14}C was found in the blood.

In thin-layer chromatography (*7*) (Fig. 1), the region of highest radioactivity in the ether extract corresponds to the area with the same R_F as α-nepetalinic acid, and one of the minor regions corresponds to the area with an R_F similar to that of dihydronepetalactone. Upon conversion of the compounds to their trimethylsilyl (TMS) derivatives (*8*), R_F values for the TMS-nepetalinate derivative and one of the compounds in the derivatives from the ether extract were identical.

Results of gas liquid chromatographic analysis (*5*) of the acid-ether urine extract from a cat fed *cis,trans*-

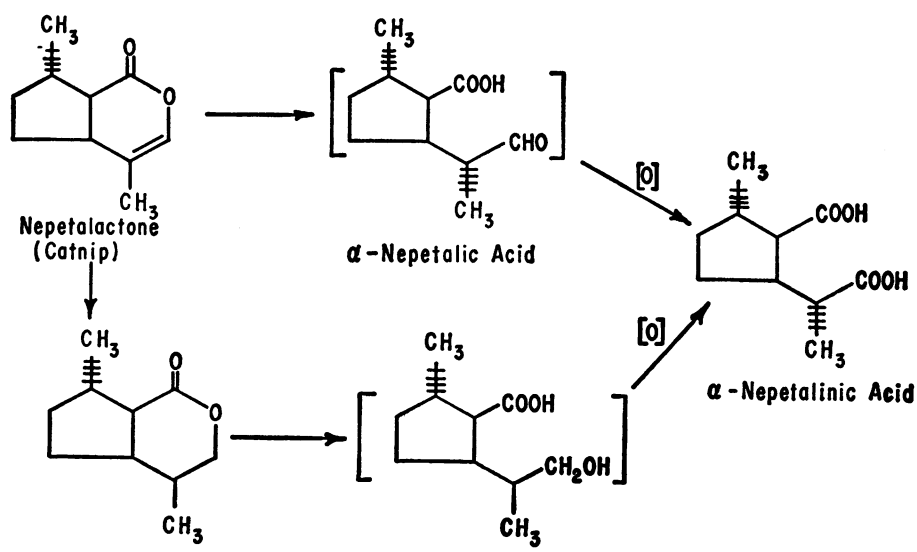

Fig. 2. Proposed route for the metabolism of *cis,trans*-nepetalactone by the cat.

nepetalactone-^{14}C showed a large peak with a retention time of 60 minutes; this peak was absent in the chromatogram of a control urine. Methylation with diazomethane (*4*) caused a change in retention time of the predominant peak, an indication that the major metabolite was a carboxylic acid. Further analysis (including chromatography with standard dimethyl nepetalinate) on Carbowax or Apiezon L indicated that the retention time of dimethyl nepetalinate and the methylated unknown were identical. Radio-gas chromatography showed that the major radioactive peak was identical with the peak detected by the hydrogen flame ionization detector and mass spectrometry. Other radioactive peaks corresponded to retention times of unreacted *cis,trans*-nepetalactone peaks, dihydronepetalactone, and an unidentified compound.

Mass spectra obtained with the combination gas chromatograph–mass spectrometer (*9*) after the extract from urine was methylated indicated the presence of α-dimethyl nepetalinate, dihydronepetalactone, and unreacted *cis,trans*-nepetalactone.

A sample of the ether extract from urine was subjected to silicic acid chromatography (*10*) and gave the same elution volume as free α-nepetalinic acid (9 ml). Direct comparison with authentic α-dimethyl nepetalinate after methylation and analysis by gas-liquid chromatography concluded identification.

The specific activity of the recovered α-dimethyl nepetalinate was 1.85×10^4 mc/mmole as compared to 1.87×10^4 mc/mmole for the administered uniformly labeled *cis,trans*-nepetalactone-^{14}C, indicating no significant endogenous dilution. Although the extent of formation varied among cats, the free α-nepetalinic acid was always quantitatively the major metabolite (50 to 75 percent).

A probable pathway for the metabolism of nepetalactone by the domestic cat is shown in Fig. 2. Two routes are proposed: (i) the direct delactonization of nepetalactone to yield α-nepetalic acid which is subsequently oxidized to α-nepetalinic acid, and (ii) the hydrogenation of nepetalactone to yield dihydronepetalactone (*11*) followed by delactonization and oxidation to yield α-nepetalinic acid (*12*). Although α-nepetalic acid is implicated as a key intermediate in nepetalactone metabolism, the available evidence to support this view is inconclusive.

Our results provide the first evidence for the metabolism of an attractant by a higher animal. It should be pointed out that the metabolism of milligram quantities of attractant may bear no relationship to the few molecules required to stimulate the olfactory receptors and produce the psychopharmacological effects.

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References and Notes

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3. Some *Nepeta cataria* plants produce *cis-trans*-nepetalactone and *trans,cis*-nepetalactone in the ratio of 99.9 to 0.1, whereas in others a ratio of 70 to 30 may be found. These isomers are difficult to separate; for the preparation of *cis,trans*-nepetalactone-^{14}C which had a minimum of *trans,cis*-nepetalactone-^{14}C, a single leaf of several plants was analyzed to locate plants producing predominantly *cis,trans*-nepetalactone; these plants were then used in the preparation of *cis,trans*-nepetalactone-^{14}C from $^{14}CO_2$.
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5. To make certain that the radioactivity collected in the NaOH traps was not respired as organic volatile compounds, a trap (—78°C) was inserted between the metabolism chamber and the first NaOH trap in the experiment with the adult female cat. The aqueous solution recovered from the traps was devoid of radioactivity; thus the respired radioactivity was $^{14}CO_2$.
6. All of the final radioactivity was recovered in the ether extract of the steam distillate. Since this represented only a small portion of the administered dose, it was not studied further.
7. Analytical thin-layer chromatography was performed on thin-layer plates (20 by 20 by 0.4 cm or 20 by 6 by 0.6 cm) coated with silica gel G (25 mg/cm^2) and developed in a hexane, acetone, ethanol (40 : 10 : 4 by volume) system. The compounds were located by staining with iodine vapor. For chromatography of the free α-nepetalinic acid a benzene, methanol, glacial acetic acid (90 : 16 : 8 by volume) system was used. After air-drying until no acetic acid could be detected by smell, the plate was sprayed with a solution of 0.04 percent bromcresol green in ethanol. The acid spots appeared yellow on a blue background. Thin-layer chromatography plates were assayed for radioactivity by (i) external scanning of the plate with a Nuclear-Chicago model 10324π Actigraph III and (ii) scraping the plates clean at appropriate areas and counting by liquid scintillation spectrometry (Tri-Carb model 314).
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11. Our evidence suggests that this is α-dihydronepetalactone based on gas-liquid chromatography retention times.
12. The specificity of the enzyme-catalyzed reactions involved in (i) opening of the lactone and (ii) oxidation to yield α-nepetalinic acid is recognized. Chemical opening of the lactone ring followed by oxidation yields approximately equal amounts of the α and δ isomers of nepetalinic acid [E. J. Eisenbraun and S. M. McElvain, *J. Amer. Chem. Soc.* **77**, 3383 (1955)]. If these reactions were the result of chemical catalysis brought about by the alkaline conditions of the small intestine, approximately equal amounts of the α and δ isomers of nepetalinic acid would be formed. No δ-nepetalinic acid was found in cat urine; all four isomers, α, β, γ, and δ, of nepetalinic acids can be distinguished based on their difference in retention times (*4*); consequently, we concluded the stereospecific oxidation of nepetalactone results from the action of mammalian enzymes.
13. Supported in part by NSF grant GB5607. We thank Dr. E. J. Eisenbraun for α-nepetalinic acid (2-carboxy-α,3-dimethylcyclopentaneacetic acid) and for helpful discussions; Dr. R. Panciera for necropsy examinations and Dr. B. L. Glenn for assistance in maintaining the cats. G.H.P. was an NDEA fellow (1965–67).

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Collagen Gels: Design for a Vitreous Replacement

Abstract. *Clear, stable gels have been prepared from purified tropocollagen from calf skin; the collagen was solubilized with a proteolytic enzyme (Proctase) and stabilized by ultraviolet irradiation under nitrogen. These gels are clear, possess altered immunologic reactivity, and have properties of an ideal vitreous replacement. Implantations in rabbit and monkey eyes appear to be well tolerated, remain clear, and gradually disappear in about 2 months.*

One of the problems in medicine and surgery today is the procurement of replacements for damaged human organs. Because of its biologic inertness and its structural stability, collagen can be altered in various ways to make it suitable for replacements. Tropocollagen, or molecular collagen, is a triple helical fibrous protein with nonhelical appendages that are critical determinants of its molecular interaction (*1*) and immunologic properties (*2*). Modification of these regions by proteolytic enzymes and by ultraviolet light leads to alterations in collagen which render it suitable for heteroimplantation. Collagen films implanted in rabbit corneas (*3*) have been in place for more than 2 years with no untoward reaction. We now report on the replacement of vitreous with specially prepared collagen gels. The human vitreous humor is a clear gel-like structure located in the posterior part of the eye. In cases of a damaged vitreous, replacement with a clear gel could help restore sight.

Collagen is extracted from calf skin by treating washed collagen fiber with Proctase (*4*), a proteolytic enzyme with