Behavioral and Toxicological Studies of Cyclopentanoid Monoterpenes From *Nepeta cataria*

JOHN W. HARNEY[1], IVAN M. BAROFSKY[2], and JOHN D. LEARY[3*]

Pharmacognosy Research Laboratory and Department of Behavioral Science, Massachusetts College of Pharmacy, Boston, Massachusetts 02115

ABSTRACT.—Two samples of catnip oil were analyzed by tlc, gc, and hplc; the results indicated the presence of 23 components. Fractionation of the commercial sample of catnip oil by either distillation or gc yielded 40% nepetalactone and 43% nepetalic acid. Catnip oil, nepetalic acid, and a nepetalactone-enriched fraction were evaluated for toxicological and behavioral effects in mice and rats. The LD_{50} of catnip oil, the nepetalactone-enriched fraction, and nepetalic acid were found in mice to be: 1300 mg/kg, 1550 mg/kg and 1050 mg/kg, respectively.

Catnip oil (500 mg/kg) and nepetalic acid (62.5 mg/kg) were found to significantly increase hexobarbital sleeping time in mice. Rats trained on a Sidman avoidance schedule showed a significant decrease in performance following intraperitoneal injections of catnip oil (500–750 mg/kg), nepetalic acid (125–250 mg/kg), and the nepetalactone-enriched fraction (500–750 mg/kg). Rats trained on the same avoidance schedule developed behavioral tolerance after daily injections of 750 mg/kg catnip oil.

Nepeta cataria L. was first described in 1753, and has been recognized for its medicinal activity by the official American compendia since 1842; it was last acknowledged in 1950. It has been described as a carminative, stimulant, diaphoretic and tonic (1). The activity of the plant has been ascribed to its volatile constituents (2).

Freshly harvested flowering tops of catnip yields 0.3 to 1.0 per cent of a volatile oil by steam distillation (3, 4, 5). The chemistry of the volatile oil of catnip and related species of *Nepeta* (*N. citriodora, N. mussini,* and *N. leucophylla*) has been extensively studied (3, 4, 6–13). The major constituent of the freshly prepared catnip oil is nepetalactone (up to 78%) (12), a cyclopentanoid monoterpene related to the iridoid compounds. Studies have been done on the biosynthetic routes leading to the formation of nepetalactone and other cyclopentanoid terpenoid substances (14–17).

Jackson and Reed (18) reported in 1969 that the catnip plant was being used as a substitute for marihuana; this original report has been followed by reports by other workers who have mentioned catnip as possibly a consciousness-altering substance (19–20). The reported effects associated with the use of catnip were that it made the user appear "happy, contented, and intoxicated"; several subjects also reported "visual and auditory hallucinations" (18).

Previous experiments have shown that catnip and its constituents have behavioral activity in felines which are dependent upon the route of administration (2, 21, 22). The purpose of the present study was to employ standard animal models to investigate the behavioral and toxicological properties of catnip oil, nepetalactone and nepetalic acid.

EXPERIMENTAL[4]

SOURCE OF PLANT MATERIAL.—Samples of catnip oil obtained by steam distillation were obtained commercially[5] and from the University of Nebraska.[6] The nepetalactone-enriched sample, which was obtained from Oklahoma State University,[7] was isolated by Waller by a combination of preparative tlc and gc (12), and was approximately 90% pure.

[1]Present Address: Surgery Branch, National Cancer Institute Bethesda, Maryland 20014.
[2]Present Address: Dept. of Anatomy, Tufts University, School of Medicine, Boston, MA 02111.
[3]Reprint requests.
[4]Melting points were taken on a Meltemp apparatus (uncorrected). Hplc, Waters model ALC 202/201, 6000 psi, with dual detectors. Perkin-Elmer gas chromatograph, model 3920 B. Spectra were determined with a Perkin-Elmer 457A integrating infrared spectrophotometer (polystyrene calibration at 1601 cm^{-1}) and Varian nmr spectrometer, model T–60, with $CDCl_3$ and D_2O.
[5]Fritzsche, Dodge and Olcutt, New York, 10011. Lot AB 7723.
[6]Dr. S. Stohs, University of Nebraska, Omaha, Nebraska, 68105.
[7]Dr. G. R. Waller, Oklahoma State University, Stillwater, OK, 74074
*A former graduate student of Dr. Arthur E. Schwarting.

FRACTIONATION OF CATNIP OIL.—The commercial sample[2] of catnip oil (10 g) was chromatographed on neutral alumina (200 g, 80–200 mesh, Fisher), and was successively eluted with 200 ml fractions of hexane, hexane-ethyl acetate mixtures (9:1; 8:2; 6:4; and 5:5), ethyl acetate, and methanol (table 1). The hexane-ethyl acetate (6:4) fractions (897 mg) were rechromatographed on neutral alumina (90 g), and the elution sequence was repeated (50 ml fractions). The hexane-ethyl acetate (6:4) fraction was concentrated *in vacuo* to yield a solid residue (124 mg) which was recrystallized from hexane-ethyl ether repeatedly; mp 76°, reported (6), 75–76°. The sodium bisulfite addition product was prepared as described by McElvain (6), mp 95–96°; reported, 95–97°.

TABLE 1. *Column chromatography of catnip oil.*[a]

Solvent	Fraction number	Weight of residue, g
Hexane	1–2	0.199
Hexane-EtOAc 9:1	3–4	0.346
Hexane-EtOAc 8:2	5–6	0.863
Hexane-EtOAc 6:4	7–12	0.897
Hexane-EtOAC 5:5	13	0.066
EtOAC	14–15	0.413
Methanol	16	3.696

[a]Commercial sample, 10.0 g; Fritzsche, Dodge and Olcutt.

Nepetalic acid was examined by ir, max (KBr): 3250, 2975, 1660, 1630, 1397, 1151, 852 cm^{-1}; pmr, δ ($CDCl_3$) 1.2 (d, CH_3–CH). 1.67 (s, CH_3–C=C), 5.67 (m, –CH–OH), 7.8 (broad, –OH). The spectrum of nepetalic acid in D_2O was similar with the following exceptions: δ 5.67 collapsed to a singlet, and δ 7.8 disappeared.

CHROMATOGRAPHY.—Tlc was carried out on silica gel GF_{254} (Merck) layers, 0.250 mm, on glass plates. Plates were developed with either hexane:acetone:ethanol (40:10:4), or benzene: ethyl acetate (2:1). Revelation was achieved by uv (blue spots on fluorescent green background) and by iodine vapors. Catnip oil (8 spots), nepetalactone (2 spots), nepetalic acid (1 spot).

Samples of oil, nepetalic acid, or nepetalactone were analyzed by hplc on Corasil II (37–50 μ, Waters) column (2 mm x 244 cm). The isocratic eluting solvent mixture was iso-octane: tetrahydrofuran (95:5); flow rate, 0.51 ml/min. Sample size was 1 μl of liquid; nepetalic acid was dissolved in methanol. Detection was by uv at 254 nm. Catnip oil yielded 23 peaks with nepetalactone and nepetalic acid as the major components; the nepetalactone-enriched fraction showed one major component and four minor peaks; nepetalic acid showed one peak.

Similar studies were carried out on 1 μl samples by gc. Samples were injected onto glass columns (1.83 m x 0.33 cm) packed with 3% OV–1, or OV–17, on Chrom–W–HP. The carrier gas was nitrogen, 30 ml/min. The column temperature was programmed from 80° to 260°; inlet temperature, 180°; FID temperature, 260°. Catnip oil showed 27 peaks; nepetalactone had one major peak and six minor peaks; nepetalic acid had one peak.

ANIMAL STUDIES.—Male albino mice (20–35 g, CD strain[8]), housed 8 per cage, were used for the determination of toxicity and for the hexobarbital sleeping time experiments. Male albino rats (200–250 g at the time of arrival at the laboratory, Sprague-Dawley, CD strain[8]) housed individually were used in the avoidance performance and tolerance experiments. All animals were maintained in an environment with a relatively constant temperature (21–24°) on a light-dark (12/12 hr) cycle and were provided with food and water *ad libitum*.

TOXICITY STUDIES.—Samples of catnip oil[5] (100–2000 mg/kg), nepetalic acid (500–1500 mg/kg) and nepetalactone-enriched sample[7] (100–2000 mg/kg) were each dissolved in isopropyl myristate for ip injection (0.1 ml/10 g of animal weight). Mice were housed eight per cage until dosed with the drug or the control vehicle. They were then transferred to plexiglas chambers (7.5 x 60 x 45 cm) and maintained individually until they were either sufficiently recovered to return them to their home cage or until death occurred. Ten animals were used at each dose. Animals were monitored for a period of 48 hr. The results are shown in table 2.

HEXOBARBITAL SLEEPING TIME POTENTIATION.—Groups of 10 mice were injected ip with either catnip oil[6] (125–1250 mg/kg) or nepetalic acid (31.25–125 mg/kg), and were placed individually into plexiglas chambers for 30 min. They were then injected ip with hexobarbital[9] (100 mg/kg) in 0.9% saline solution. Loss of righting reflex was determined by placing a mouse

[8]Charles River Breeding Laboratories, Wilmington, Mass. 01887.
[9]Winthrop Laboratories, New York, 12144.

TABLE 2. TOXICITY OF CATNIP OIL, NEPETALACTONE AND NEPETALIC ACID.[a]

Drug	LD_{50}	Minimal Lethal dose[b]	Minimal Dose for Loss of Righting Reflex[b]
Catnip oil	1300 mg/kg	1000 mg/kg	1000 mg/kg
Nepetalactone	1550 mg/kg	1500 mg/kg	1000 mg/kg
Nepetalic Acid	1050 mg/kg	500 mg/kg	250 mg/kg

[a]Observations were made on groups of ten mice at each dose level.
[b]Values reported for minimal lethal dose and minimal dose for loss of righting reflex are those at which at least one animal showed the response noted.

on its back, and if it failed to right itself within 5 sec, it was defined as having lost its righting reflex. The ability of the mice to turn from the dorsal to the ventral side without the aid of the observer was considered to be regaining its righting reflex. Sleeping time is the period between loss of, and return of, the righting reflex. The results are shown in fig. 1.

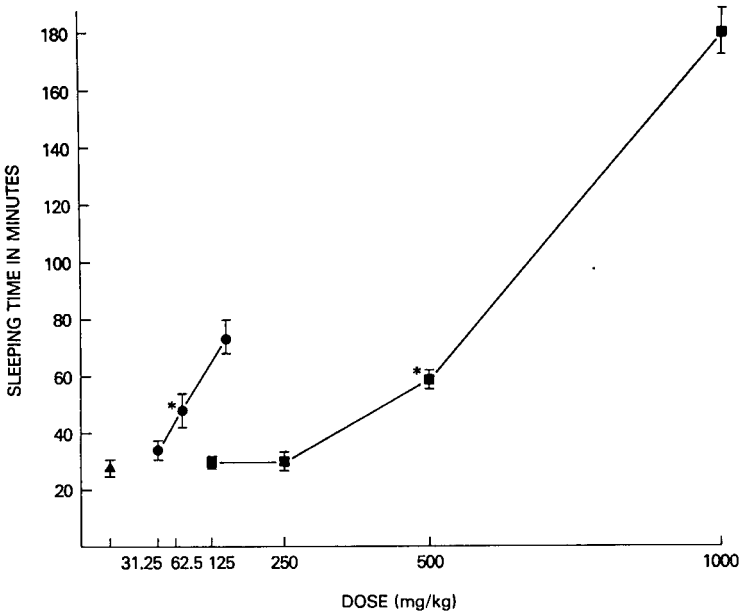


FIG. 1. *Hexobarbital Sleeping Time.*

Represents the mean values (± S.E.M.) for 10 mice at each point.

Hexobarbital sleeping time control (▲), hexobarbital+catnip oil (■), hexobarbital+nepetalic acid (●).

*Indicates the lowest dose at which there was a significant increase above control hexobarbital sleeping time ($p<0.01$, Student's t-test).

SIDMAN AVOIDANCE PERFORMANCE STUDY.—Rats were trained and tested in standard operant conditioning chambers (floor of stainless steel rods for presentation of a scrambled foot shock and a lever to allow delay of shock presentation). The parameters of the Sidman avoidance schedule are: shock to shock interval of 5 sec, response to shock interval of 15 sec; the shock intensity of 2 mAMP had a duration of 0.5 sec. Rats were trained until a stable baseline performance was obtained (minimum of 50 hr). Experimental sessions lasted 255 min: initial observation of inter-session baseline performance (60 min), injection period (drug or placebo) (15 min), followed by drug or control observation period (180 min). Drug and control sessions were alternated every other day, or drug presentation was delayed until the animal's performance returned to baseline. Catnip oil[6] (125–750 mg/kg), nepetalic acid (62.5–250 mg/kg) and the nepetalactone-enriched fraction (62.5–750 mg/kg) were given ip in isopropyl myristate (0.1 ml/100 g). The data are given in figs. 2a, 2b, and 2c.

The effect of *catnip oil* on Sidman avoidance performance.

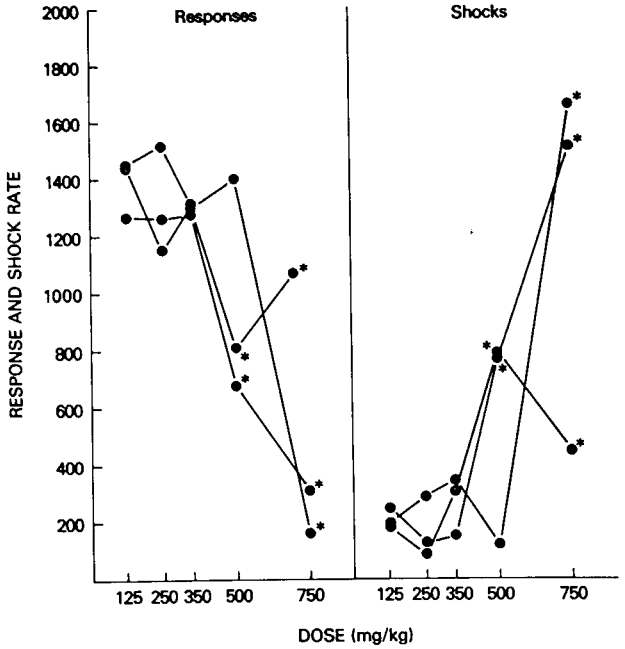


FIG. 2a.

The effect of *nepetalic acid* on Sidman avoidance performance.

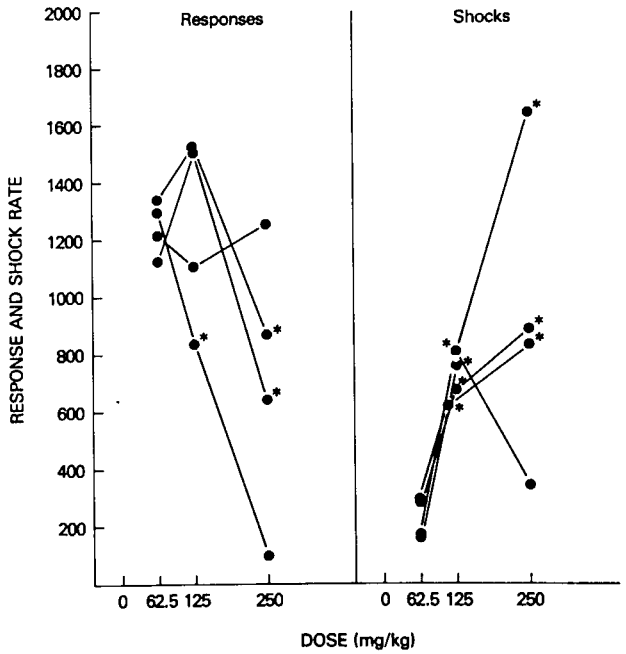


FIG. 2b.

The effect of *nepetalactone* on Sidman avoidance performance.

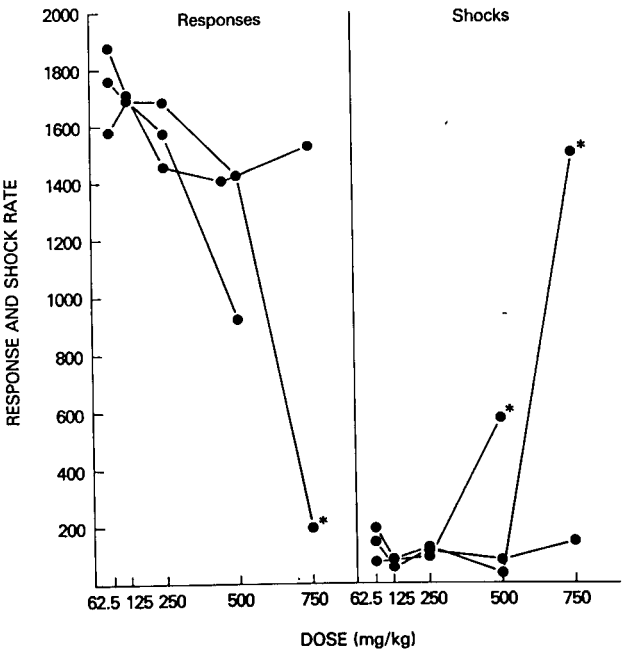


FIG. 2c.

FIGS. 2a, 2b and 2c. *Sidman Avoidance Study.*

2a. Catnip oil, 2b. Nepetalic acid, 2c. Nepetalactone

Represents response and shock rates for individual animals.

*Indicates points at which the response or shock rate, under drug conditions, fell more than two standard deviations away from the pooled control data of that particular animal.

TOLERANCE STUDY.—Six naive rats were trained on the Sidman avoidance protocol, described earlier, for a minimum of 50 hr. The animals were then run in a series of at least seven control sessions, following the initial training sessions, to obtain a measure of their baseline performance. Animals were observed in two hour experimental sessions. The first hour served as a measure of intersession performance. The animals were interrupted for 15 min to be weighed and injected, which was followed by a one hour drug-observation session. Catnip oil[6] in isopropyl myristate (750 mg/kg) was given ip for 10 consecutive days. An increase in response rate or a decrease in shock rate (return to baseline performance) following catnip injection would indicate development of behavioral tolerance to the effects of the catnip oil. The data are presented in figure 3.

RESULTS AND DISCUSSION

Analysis of two samples of catnip oil by tlc, gc and hplc indicated a complex mixture of at least twenty-three components. The commercial sample of oil was subjected to fractionation on neutral alumina columns into sixteen fractions (table 1). The hexane-ethyl acetate (6:4) fractions yielded one major crystalline product, which was recrystallized from a mixture of hexane-ethyl ether according to the procedure of McElvain and coworkers (6). This compound, mp 76°, was converted to the bisulfite adduct, mp 95–96°, which agreed with published data (6) for nepetalic acid. Comparison of the physical appearance of the solid and chromatographic patterns (tlc, gc, hplc) of this product with

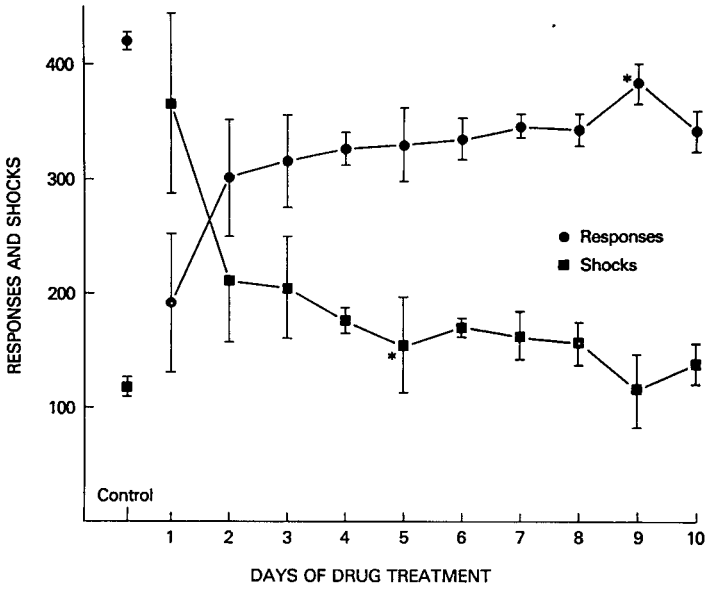


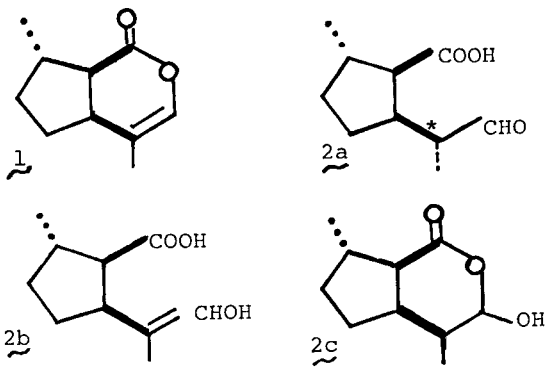
FIG. 3. *Behavioral Tolerance.*

Represent the mean (± S.E.M.) response rate (●), and shock rate (■) for one hour observation sessions following ip injection of catnip oil (750 mg/kg).

Control data represent a pool of data from 20 control sessions for for each animal.

*Indicates the day on which there was no different in either the shock or response rate between control session and drug sessions ($p > 0.05$, Student's t-test).

nepetalactone **1** supplied by Dr. Waller verified that the two products were not identical. The ir and pmr spectra confirm the absence of lactone function in the isolated nepetalic acid and suggest structure **2** as the crystalline substance used in these studies. It is presumed that the high yield of nepetalic acid in this sample was attributed in part to the age of the sample. Chemical studies of the freshly steam-distilled flowering tops of the catnip plant and related species of *Nepeta* yield up to 78% of the bicyclic monoterpene nepetalactone **1**; isomers of nepetalactone, dihydronepetalactones, nepetalic acid **2**, as well as acyclic terpenes (citronellal, geraniol, citral), have also been reported (8, 12). The



chemistry of catnip constituents and other cyclopentanoid-containing natural products have been reviewed by Cavill (24).

Iridoid substances are well known as behaviorally-active substances to both insects (24, 25) and to cats (2, 5, 21–23). Many members of the feline family respond to catnip plants, the oil and nepetalactone. Biological studies in other animals is less definitive. Initial studies were concerned with determining the toxicity of catnip oil, nepetalic acid, and a nepetalactone-enriched fraction. The toxicity studies were carried out in mice, and the data are summarized in table 2. The range of responses was similar for each substance tested; in all instances, animals responded to nepetalic acid at a lower dose than to either the catnip oil or the nepetalactone-enriched fraction. The most prominent changes observed in behavior were a reduction in overall activity, ataxia, and the loss of righting reflex at higher doses. Because the observations of gross behavior during the toxicity studies suggested that the substances might be showing a depressant type of action, an investigation of their effects on hexobarbital sleeping time was done.

The effects of catnip oil and nepetalic acid on hexobarbital sleeping time are shown in fig. 1. Sleeping time was significantly increased ($p < 0.01$ by Student's t-test) above that of the control group of animals by a dose of 62.5 mg/kg of nepetalic acid; however, the sleeping time was not significantly increased by catnip oil until a dose of 500 mg/kg was reached.

The effects of catnip oil, nepetalic acid, and the nepetalactone-enriched fraction on avoidance performance are shown in fig. 2 (a, b, c). The data plotted are for individual animals. A significant effect on avoidance behavior was considered to be that dose that produced a shock or response rate equivalent to, or greater than, two standard deviations away from the mean of each animal's control performance. As can be seen, nepetalic acid (125 mg/kg) had effects on both response and shock rate at lower doses than either catnip oil (500 mg/kg) or the nepetalactone fraction (500–750 mg/kg).

The data obtained from the tolerance study are presented in fig. 3. The data from one of the six animals was excluded from the figure because it died during the course of the drug treatment. It can be seen that initially the catnip oil (750 mg/kg) disrupted the animals' performance; but by the fifth day of drug treatment, the shock rate approached the baseline level ($P > 0.05$ by Student's t-test). The response rate returns to baseline only on the ninth day of drug treatment. The return to baseline performance (lowered shock rate and increased response rate) is interpreted as the development of behavioral tolerance.

These experiments in mice and rats have shown that catnip oil, nepetalactone, and nepetalic acid can be behaviorally active in animal species other than felines and insects, albeit at large doses. The observations made during the toxicity and hexobarbital sleeping time studies suggest that, when given intraperitoneally, these substances elicit effects of a depressant nature. It should be noted, however, that when catnip oil or nepetalactone is administered to cats *per os*, or via the ip route, typical feline response is absent (2); olfactory administration is needed to produce the typical feline reaction (22, 23). The abuse potential of these substances would be expected to be low due to the large doses required to obtain effects, and the correspondingly low concentrations of these substances in the catnip plant.

ACKNOWLEDGMENTS

We wish to thank Dr. G. Constantine for a sample of nepetalactone. Special thanks are extended to Dr. G. Waller for the generous amounts of nepetalactone used in these studies and to Dr. S. Stohs for generous amounts of catnip oil. We also express our thanks to Dr. Williams and Dr. Kelley for spectral data interpretation and to Mr. W. Dark (Waters Associates) for hplc advice.

Received 10 April 1978.

LITERATURE CITED

1. BENTLY, R., and H. TRIMEN. 1880. *Medicinal Plants*. Vol. III. Monograph No. 209, J. and A. Churchill, London.
2. WALLER, G. R., G. H. PRICE, and E. D. MITCHELL. 1969. Feline attractant *cis,trans*-nepetalctone: Metabolism in the domestic cat. *Science* **164**: 1281–1282.
3. HIXON, R. 1922. Oil of catnip. *J. Am. Pharm. Assoc.* **11**: 96–98.
4. MEYER, M. 1936. The crystalline acid from catnip oil. *Pharm. Arch.* **7**: 17–52.
5. GUENTHER, E., and D. ALTHAUSEN. 1949. *The Volatile Oils*, Vol. II, D. Van Nostrand Co., Inc., New York. pp. 434–436.
6. MCELVAIN, S. M., R. D. BRIGHT, and P. R. JOHNSON. 1941. The constituents of the volatile oil of catnip. I. Neptalic acid, nepetalactone, and related compounds. *J. Am. Chem. Soc.* **63**: 1558–1563.
7. MCELVAIN, S. M., P. M. WALTERS, and R. D. BRIGHT. 1942. The constitutents of the volatile oil of catnip. II. The neutral components. Nepetalic anhydride. *J. Am. Chem. Soc.* **64**: 1828–1831.
8. MCELVAIN, S. M., E. J. EISENBRAUN. 1955. The constitutents of the volatile oil of catnip. III. The structure of nepetalic acid and related compounds. *J. Am. Chem. Soc.* **77**: 1599–1605.
9. MEINWALD, J. 1954. The degradation of nepetalactone. *J. Am. Chem. Soc.* **76**: 4571–4572.
10. EISENBRAUN, E. J., and S. M. MCELVAIN. 1955. The configurations of the 3-methylcyclopentanones and related compounds. *J. Am. Chem. Soc.* **77**: 3383–3384.
11. REGNIER, R. E., E. J. EISENBRAUN, and G. R. WALLER. 1967. Nepetalactone from *Nepeta cataria* L. *Phytochemistry* **6**: 1271–1280.
12. REGNIER, R. E., G. R. WALLER, and E. J. EISENBRAUN. 1967. Studies on the essential oil of three *Nepeta* species. *Phytochemistry* **6**: 1281–1289.
13. SASTRY, S. D., W. R. SPRINGSUBE, and G. R. WALLER. 1972. Identification of 5,9-dehydronepetalactone. A new monoterpene from *Nepeta cataria*. *Phytochemistry* **11**: 453–455.
14. MEINWALD, J., G. R. HAPP, J. LABOW, and T. EISNER. 1966. Cyclopentanoid terpene biosynthesis in a phasmid insect and catmint. *Science* **151**: 79–80.
15. MITCHELL, E. D., M. DOWNING, and G. R. GRIFFITH. 1972. $^{14}CO_2$ Incorporation into nepetalactone. *Phytochemistry* **11**: 3193–3194.
16. AUDA, H., H. R. JUNEJA, E. G. EISENBRAUN, G. R. WALLER, W. R. KAYS, and H. H. APPEL. 1967. Biosynthesis of methylcyclopentane monoterpenoid I. *Skytanthus* alkaloids. *J. Am. Chem. Soc.* **89**: 2478–2482.
17. DOWNING, M. R., and E. D. MITCHELL. 1974. Metabolism of mevalonic acid to phosphorylated intermediates in a cell-free extract from *Nepeta cataria* leaves. *Phytochemistry* **13**: 1419–1421.
18. JACKSON, B., and A. REED. 1969. Catnip and the alteration of consciousness. *J.A.M.A.* **207**: 1349–1350.
19. EMBODEN, W. A. 1972. *Narcotic Plants. Hallucinogens, Stimulants, Inebrients and Hypnotics—Their Origins and Uses*. The MacMillan Comp., New York, p. 20.
20. GERSTAD, G. 1972. Naturally occurring hallucinogens. III. *Quart. J. Crude Drug Res.* **12**: 1846–1849.
21. HATCH, R. C. 1972. Effect of drugs on catnip (*Nepeta cataria*) induced pleasure behavior in cats. *Am. J. Vet. Res.* **33**: 143–155.
22. HAYASHI, T. 1969. Motor reflexes of cats to *Actinidia polygama* (Japan) and to catnip (U.S.A.). *Theor. Odor Meas., Proc.* 351–358; *Chem. Abst.* **71**: 122223m.
23. HAYASHI, T. 1969. Pseudo-affective reflexes from olfaction in decorticated cats. *Abh. Deut. Akad.* **2**: 101–102; *Chem. Abstr.* **67**: 20247m.
24. CAVILL, G. W. K. 1969. *Insect Terpenoids and Nepetalactone, in Cyclopentanoid Terpene Derivatives*. Eds. TAYLOR, W. I., and A. R. BATTERSBY. Marcel-Dekker, Inc., New York, pp. 203–238.
25. CAVILL, G. W. K., and P. L. ROBERTSON. 1965. Ant venoms, attractants and repellants. *Science* **149**: 1337–1345.
26. COLTHRUP, N. B., L. H. DALY, and S. E. WIBERLEY. 1964. *Introduction to Infrared and Raman Spectroscopy*. Academic Press, New York, pp. 244–248.