



Cyclopentanoid Terpene Biosynthesis in a Phasmid Insect and in Catmint

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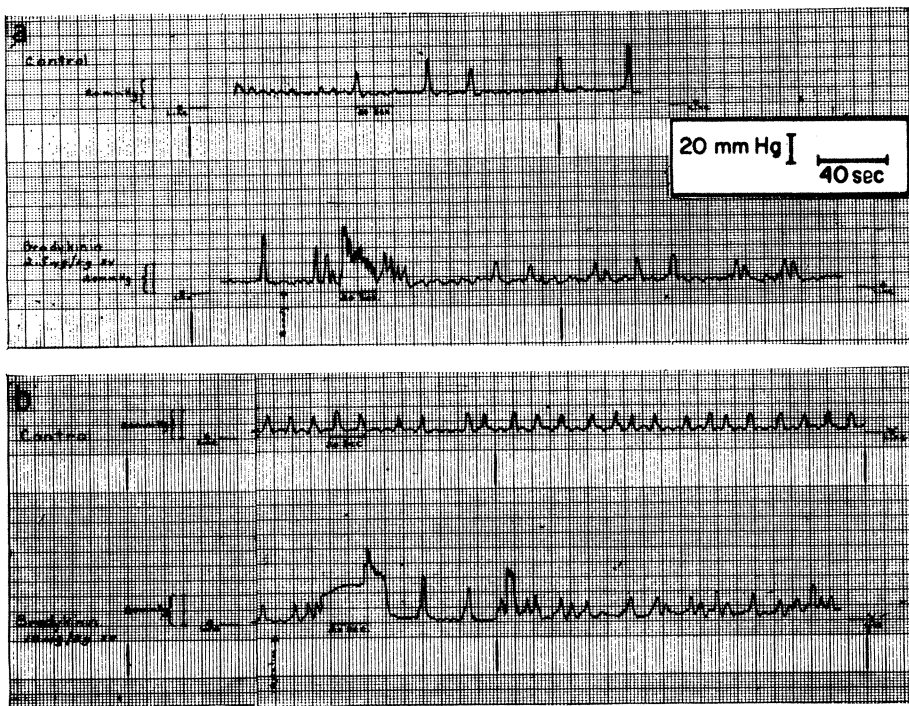


Fig. 1. The effect of bradykinin on ureteral peristaltic pressures in dog with explanted bladder: (a) 2.5 $\mu\text{g}/\text{kg}$ intravenously; (b) 10 $\mu\text{g}/\text{kg}$ intravenously.

at intervals of 1 minute for the first 5 minutes and during the 12th and 17th minute after the administration of an estimated 2.5, 5.0, and 10.0 $\mu\text{g}/\text{kg}$. There was significant diuresis or anti-diuresis. In all the ureters the urine flow was between 0.05 and 0.30 ml per minute.

The effect of bradykinin upon ureteral perfusion was similar to that of histamine or serotonin. In two experiments on one dog, the rate of flow by ureter from a reservoir 14 and 30 cm above the renal pelvis was depressed from 24 drops per minute to 7 and 8 drops per minute after the administration of 1.4 $\mu\text{g}/\text{kg}$ of bradykinin. The effect lasted 2 minutes after the first dose and slightly longer after the second dose.

Two rats (200 g) were anesthetized with ether, and the abdominal cavity was opened. The animals were given 25 or 50 μg of bradykinin per kilogram of body weight. Frequency of ureteral peristalsis increased for 2 to 4 minutes. After the largest dose (100 $\mu\text{g}/\text{kg}$), ureteral contractions occurred that were unaccompanied by a urinary bolus. With larger doses dyskinesias, with to-and-fro peristalsis and irregularities of peristalsis, appeared for a short period.

Bradykinin can be grouped with histamine and serotonin as a ureteral stimulant. Certain antihistamines in large

doses act as weak histamine agonists, stimulating the ureter (2). Histamine, serotonin, and bradykinin have all been implicated in the cellular response to injury, inflammation, or allergy and all three have potent extracellular antagonists (6).

The enzymatic destruction of bradykinin explains its evanescent effect (9). Discovery of the ureteral activity by bradykinin sheds light on ureteral contractility by demonstrating that a non-peptide is active.

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Cyclopentanoid Terpene Biosynthesis in a Phasmid Insect and in Catmint

Abstract. The stick insect, *Anisomorpha buprestoides*, and the catmint, *Nepeta cataria*, produce closely related cyclopentanoid terpenes, anisomorphal and nepetalactone. Tracer experiments with isotopes indicate that anisomorphal is synthesized by the walking stick from normal terpene precursors (acetate or mevalonate). In the catmint plant, isolated leaf disks synthesized nepetalactone, utilizing the same precursors.

Cyclopentanoid monoterpenes have been found in both plants and animals (1). Although not a very large group of compounds, they are beginning to attract increased attention (2). A monocyclic representative of this group, anisomorphal (I) (3), has recently been shown to serve a defensive function in the Southern walking stick, *Anisomorpha buprestoides* (4). The best studied of these compounds is nepetalactone (II) (5), long known for its bizarre effect on feline behavior (6), and recently shown to have a possible protective role for the catmint which produces it (7). We have been interested in the general question of the origin of repellent compounds in the defensive secretions of arthropods and have recently reported on the biosynthesis of two acyclic terpenes, citronellal (III) and citral (IV), in an ant (8). Plausible ionic (9) and photochemical (10) pathways have been suggested for forming cyclopentanoid terpenes from acyclic precursors such as III and IV; we now report exploratory biosynthetic experiments for the insect terpene I, along with parallel results for nepetalactone, II (11).

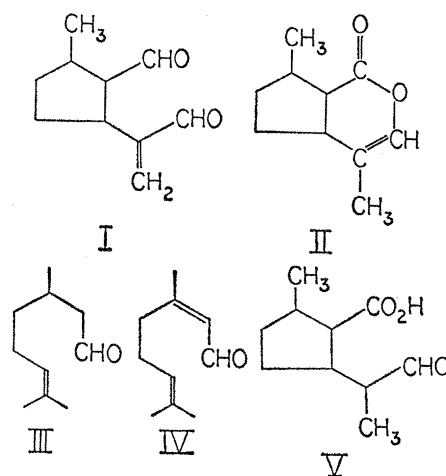


Table 1. Anisomorph biosynthesis from precursors indicated; dpm, disintegrations per minute.

Dose (mg)	Activity (dpm)	I-bis-2, 4-DNP (dpm)	Incorporation (%)
0.14	1.65×10^8	6.10×10^4	0.04
	<i>Acetate-1-¹⁴C</i>		
.27	2.18×10^8	1.65×10^5	.08
	<i>Acetate-2-¹⁴C</i>		
.63	1.10×10^8	6.50×10^4	.06
	<i>Malonate-2-¹⁴C</i>		
1.04	5.50×10^7	2.30×10^8	.008
	<i>DL-Mevalonic-lactone-2-¹⁴C</i>		

In the anisomorph study, labeled potential precursors were dissolved in Ringer solution, and 25- μ l portions were injected into *A. huprestoides* (female adults). The defensive secretion was collected in methylene chloride solution at 3-day intervals, and the combined secretion was treated with 2,4-dinitrophenylhydrazine reagent. The resultant anisomorph bis-2,4-dinitrophenylhydrazone was purified by thin-layer chromatography on silica gel G. Only one spot, corresponding to the authentic derivative, was radioactive. Two successive chromatograms, developed with a mixture of benzene and chloroform and chloroform alone, were usually sufficient to achieve a complete separation of the desired derivative from the excess reagent so that the resulting product had constant specific activity. This material was eluted from the chromatogram plates and assayed on stainless steel planchets; a Nuclear-Chicago gas-flow counter (model 181A), with an estimated counting efficiency of about 20 percent, was used. Table 1 summarizes some of the incorporation data and provides good qualitative evidence for the ability of this insect to synthesize I from the normal terpene precursors, acetate, malonate, and mevalonic lactone (I). These results are in good accord with

Table 2. Nepetalactone biosynthesis from precursors indicated.

Dose (mg)	Activity (dpm)	Nepetalic acid (dpm)	Incorporation (%)
0.78	1.1×10^9	1.2×10^5	0.01
	<i>Acetate-1-¹⁴C</i>		
.35	2.7×10^8	5.7×10^4	.02
	<i>Acetate-2-¹⁴C</i>		
1.1	1.6×10^7	6.2×10^3	.006
	<i>Mevalonate-2-¹⁴C</i>		

our earlier observations on acyclic monoterpene biosynthesis in the ant *Ac-anthomyops claviger* (Roger) (8), and along with the studies of Schmiialek (12) on farnesol synthesis in moths they suggest that terpene biosynthesis may occur generally in insects. The apparently greatly limited ability of these animals to make sterols (13) cannot, therefore, be attributed to an incapability of carrying out the initial steps in their biosynthesis.

In studying nepetalactone synthesis in the catmint, *Nepeta cataria*, initial experiments with intact plants which had been transferred to a culture solution (14) were disappointing. Thus, when sodium acetate-2-¹⁴C was added to the culture medium, there was good uptake of the isotope (about 95 percent), but extraction of young leaves (2.6 g) gave an oily residue (156 mg) which showed only 0.025 percent of the activity administered. Nepetalactone, isolated from this extract by thin-layer chromatography on silica gel G, showed only about 0.0002 percent of the original activity. An alternative technique, with isolated disks cut from expanding leaves, gave much better results (15). In this procedure, leaf disks (1 cm in diameter, about 10 mg each) were floated on distilled water containing the ¹⁴C-labeled precursors. In a typical experiment, three 30-ml beakers containing 2 ml of solution and four disks each were allowed to stand for 3 days before the slightly wilted disks were removed and extracted with methylene chloride. Authentic nepetalactone (~ 10 mg) was added to the dried extract as carrier, and the sample was applied to a silica gel G chromatography plate impregnated with rhodamine 6G. The plates were developed with a mixture of hexane and ethyl acetate (6:1), and showed three distinct bands under ultraviolet light, the nepetalactone having an R_f value of about 0.5. This band was eluted and chromatographed again. The resulting lactone fraction was hydrolyzed to give nepetalic acid (V) by treatment with 10 percent aqueous sodium hydroxide at room temperature for 90 minutes. The alkaline solution was acidified and extracted with ether, and the nepetalic acid (R_f ~ 0.15) purified by thin-layer chromatography as described. This acid was eluted with chloroform and was counted directly on steel planchets (Table 2). It is evident from the data that the catmint plant is able

to produce II from acetate and mevalonate, probably by a classical biosynthetic mechanism. The better incorporation of label from acetate than from mevalonate may be due to any number of factors, and it is interesting that even the uptake of the mevalonate by the leaf disks (60 percent) was much poorer than the acetate uptake (95 percent).

Although it is obvious that many intimate details remain to be clarified for these biosyntheses, this work confirms the generally assumed broad outlines for the construction of two typical cyclopentanoid terpenes, and incidentally demonstrates that isolated catmint leaf disks are capable of carrying out the entire biosynthetic process.

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