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$^{14}CO_2$ INCORPORATION INTO NEPETALACTONE*

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Key Word Index—*Nepeta cataria*; Labiatae; *cis,trans*-nepetalactone; terpene biosynthesis.

Abstract—The incorporation of $^{14}CO_2$ into the free-terpenoid fraction of *Nepeta cataria* was investigated by TLC-autoradiography. *cis,trans*-Nepetalactone was not labeled with ^{14}C until after 3 min had elapsed. Glyceric acid was labeled at 6 sec and other Calvin cycle intermediates were labeled at short times.

INTRODUCTION

DURING bioysnthesis *in vivo* of the feline attractant[1] *cis,trans*-nepetalactone from *N. cataria*, commonly called 'Catnip', mevalonic acid-2-^{14}C is incorporated into *cis,trans*-nepetalactone and other terpenoids.[2] The incorporation is 0·001–0·01%, which may be due to lack of transport of mevalonic acid or to a low rate of synthesis of the free terpenoids.

Nepeta cataria plants are of two types, those that produce both *cis,trans*- and *trans,cis*-nepetalactone and those that produce mainly the *cis,trans* isomer.[3,4] Only the latter plants were used in the present investigation.

TABLE 1. INCORPORATION OF $^{14}CO_2$ INTO HEXANE SOLUBLE EXTRACT OF *N. cataria* SEEDLINGS*

Incorporation time	dpm/g fr. wt	Incorporation time	dpm/g fr. wt	Incorporation time	dpm/g fr. wt
35 sec	536	2 min	25 200	4 min	53 200
1 min	24 800	3 min	32 800	10 min	250 000

* Sample fr. wt was 0·2–0·3 g.

RESULTS

Table 1 shows the incorporation of $^{14}CO_2$ into the hexane extracts of *N. cataria* seedlings. The hexane soluble extract of *N. cataria* contains free terpenoids, of which *cis,trans*-nepetalactone is the major component. The results show an increase in $^{14}CO_2$ incorporation per g fr. wt with time. The hexane extracts were chromatographed in hexane and then in C_6H_6–EtOAc (9 : 1). *cis,trans*-Nepetalactone has an R_f of 0·83 and standard ^{14}C-nepetalactone was co-chromatographed with the hexane extracted material. The chromatographed extracts at 35 sec, 2, 3 and 4 min show that labeling of the terpenoid fraction does not occur until

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3 or 4 min have elapsed. After 5 min, a radioactive spot of R_f 0·95 appears to have more radioactivity than nepetalactone. Also after 10 min the major labeling appears to be in nepetalactone which is consistent with it being the major constituent of the oil of catnip.

The autoradiography of the aqueous extracts using the two dimensional system of Benson *et al.*[5] shows that Calvin cycle intermediates appear within the short times with glyceric acid appearing at 6 sec. At 1 min there is labeling in the sugar phosphates and amino acids and at 3 min there is very heavy labeling of ^{14}C.

DISCUSSION

From the results in Table 1, it appears that a slight incorporation into the hexane soluble extract of *N. cataria* occurred within 35 sec; significant amounts of radioactivity were observed after one min experiments; however, 4 min were required for synthesis of nepetalactone. Chromatography and radioautography of this hexane soluble material showed radioactive material at the origin of the 35 sec, and 2 min experiments. It was not until 3 min elapsed that radioactivity was noticed in nepetalactone (R_f 0·83) and other terpenoids. At 5 min, there was radioactivity in a compound (R_f 0·92) less polar than nepetalactone.

The observation that $^{14}CO_2$ is not incorporated into nepetalactone and other terpenoids until 3 min have elapsed suggests a lag between $^{14}CO_2$ fixation in the chloroplast and the incorporation of acetate into the mevalonic pathway of terpenoid synthesis. It is logical to assume that acetate is transported to the cytoplasm since it has been demonstrated that mevalonic acid is not transported very well and that there are two mevalonic kinase enzymes,[6] one located in the chloroplast which leads to chloroplastic terpenoids and the other located outside the chloroplast which leads to the production of free terpenoids. The Calvin cycle is operative inasmuch as glyceric acid is the first product to appear. The labeling in the chloroplast occurs long before ^{14}C labeling appears in the terpenoid fraction which is consistent with the view that terpenoid synthesis is cytoplasmic and that acetate is transported from the chloroplast to the cytoplasm which leads to the mevalonic pathway.

EXPERIMENTAL

Plant material. The seeds of selected *Nepeta cataria* plants were germinated on filter paper and transferred to soil pots in a growth chamber which had a 16-hr day (humidity of 40%, temp. 33°) and 8-hr night (temp. 18°). The seedlings used for these experiments were 18–25 days old. *cis,trans*-Nepetalactone was photosynthetically prepared by administration of $^{14}CO_2$ released from 1 mCi of $Ba^{14}CO_3$ to *Nepeta cataria L.* plants.[1]

Photosynthesis and autoradiography. Young *N. cataria* seedlings 18–25 days old were removed from pots and washed. The seedlings were quickly weighed (between 0·1 and 0·3 g) and the roots were placed in H_2O. The seedlings were stored in the dark for 10 min and then placed into a rectangular plexiglass chamber 147 × 110 × 23 cm. The lid had two openings, one leading to a vacuum line and the other equipped with a side arm tube with stopcock and a rubber septum on top. The side arm contained 300 mCi $BaCO_3$ ^{14}C which was purchased from Cal Atomic (specific activity 54·5 mCi/mM). The chamber was evacuated and the side arm was injected with 1 ml 2 N H_2SO_4. The side arm was warmed by flame until effervescence was noticed and simultaneously the stopcock was opened and the stopwatch was started. At 5 sec before the elapsed time, the seedling was removed and placed into a mortar containing 1 ml hexane. The plantlets were ground and the hexane extract was centrifuged, assayed for ^{14}C by scintillation counting and chromatographed on Chomar 500 TLC sheets using hexane (15 cm) followed by C_6H_6–EtOAc (9:1) (10 cm). Autoradiograms were obtained after placing the developed TLC sheets on 20 × 25 cm GAF X-ray film for 4 weeks. For aqueous extracts of photosynthetate, two-dimensional chromatography using PhOH–H_2O and *n*-BuOH–$EtCO_2H$–H_2O[5] was applied.

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