METABOLISM OF NEPETALACTONE AND RELATED COMPOUNDS IN NEPETA CATARIA L. AND COMPONENTS OF ITS BOUND ESSENTIAL OIL

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Metabolism of [G-14C]nepetalactone by Nepeta cataria plants yielded significant amounts of [G-14C]dihydronepetalactone that were bound to plant components. Isomeric forms [G-14C]dihydronepetalactone and [G-14C]nepetadiol were synthesized from [G-14C]nepetalactone. Upon administration of synthetic [G-14C]dihydronepetalactone to N. cataria plants, label was incorporated into 4αα,7α,7αα-nepetalactone (1), 4αα,7α,7αβ-nepetalactone (2), a new nepetadiol (6), and de-4-methylnepetalactol (7). Feeding of [G-14C]nepetadiol caused incorporation of significant amounts of label into new dehydrated derivatives of nepetadiol. Analysis by GC and GC/MS showed that treatment of the plant residues with hot 2N HCl liberated four times as much steam-volatile material, which contained diastereoisomeric dihydronpetalactones. A metabolic scheme is proposed, and possible biological significance of the results is discussed.

INTRODUCTION

Nepeta cataria L., commonly known as catnip or catmint, produces the feline attractant 4αα,7α,7αα-nepetalactone (1) along with a number of other volatile compounds including 4αα,7α,7αβ-nepetalactone (2) and 4αβ,7α,7αβ-nepetalactone (3) (1, 2). Another feline attractant, 4αα,7α,7αα-dihydronepetalactone (4), has also been isolated from N. cataria (3) and its metabolism studied, while 4αα,7α,7αβ-dihydronepetalactone (5) was synthesized by catalytic reduction (4, 5). Figure 1 shows the structures of these terpenoids. The structure and stereochemistry of isomeric nepetalactones and related methylcyclopentane monoterpenoids were the subject of a recent investigation (6), and the newly established stereochemistry has been represented where possible. Compound 1 is a powerful insect repellant (7). Most studies have been carried out using the essential oil of the plant, obtained by steam distillation of the leaves and stems. This paper describes metabolic studies of N. cataria by feeding carbon-14-labeled nepetalactones (1 and 2), dihydronpetalactones (4 and 5) and nepetadiols (9) to plants and identifying metabolites after steam distillations and other separation procedures.

MATERIALS AND METHODS

Plant Material. Nepeta cataria L. plants were propagated from cuttings and grown in vermiculite-soil mixture in the Oklahoma State University Horticulture greenhouse; they were approximately 8 to 12 weeks old when the experiments were initiated.

Preparation of [G-14C]Nepetadiols. [G-14C]-Nepetalactones were prepared by allowing young Nepeta cataria plants to photosynthesize in the presence of 1 mCi of 14CO2 for 36 hr and isolating products according to the method of Regnier, et al. (8) and Mitchell, et al. (9). The specific activity of [G-14C]-1 and [G-14C]-2 purified by TLC varied from 11.6 × 10^3 dpm/mg to 20.6 × 10^3 dpm/mg. These products were not further purified but were injected into N. cataria plants and used for preparation of [G-14C]-dihydronpetalactones. Their identity was confirmed by gas.

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chromatography (GC) retention time and gas chromatography/mass spectrometry (GC/MS).

**Hydrogenation of Nepetalactones to Form Dihydronepetalactones.** Varying amounts (50-429 mg) of 1 and 2 and the carbon-14-labeled forms were placed in a thick-walled glass hydrogenation vessel with 30-50 ml of dry ethanol and 25-175 mg of palladium-on-charcoal (10%) catalyst. The vessel was then wrapped in a heating tape and the contents hydrogenated in a Parr pressure reaction apparatus at 4.5 atmospheres and 45-60 C for 36 hr. The yields of 4 and 5 were 80-95%; the product was analyzed by thin-layer chromatography (TLC), GC, and GC/MS. Previous results of such hydrogenation had indicated that 4 was the major product and 5 the minor one, the former being predominant in *N. cataria* (2). Catalytic hydrogenation produced 4 and 5 in a 90:10 ratio corresponding to the ratio of the reactant nepetalactones. Traces of the 4β isomers of 4 and 5 were detected. When 1 was used as the GC standard (retention time R_t = 1.00), 5 showed an R_t of 1.38 while 4 had the value of 1.70.

**Preparation of Nepetadiols.** The reduction of 4 and 5, as described by Regnier et al. (4), with lithium aluminum hydride was used for the preparation of [G-14C]-9. Both labeled and unlabeled 9 were purified by TLC using hexane:acetone:ethanol (40:10:4, v/v/v) or by preparative GC. The yields varied from 86-95%. The purity was established by GC/MS on the free compound and the trifluoroacetyl derivative. The 7αβ isomeric forms of nepetadiol were not detected.

**Isolation of Essential Oils.** The plants were harvested, cut into small pieces, and homogenized in a Waring Blender with deionized water. A current of steam was passed through the homogenate for several hours. The condensate was saturated with sodium chloride and shaken four times with 200-ml portions of diethyl ether. The combined ether extract was dried over anhydrous sodium sulfate and the ether removed under nitrogen gas. Thin-layer chromatography, GC, and GC/MS were carried out on the oil. The residue from the steam distillate was dried and extracted three times with methanol in Experiment I. The residue from the steam distillate was made 2N in HCl and heated for 2 hr at 90-100 C. The residue was then neutralized and treated with steam a second time and the isolation procedure and analyses repeated.

**Administration of Precursors.** [G-14C]Nepetalactones (1 and 2), [G-14C]dihydronepetalactones (4 and 5), and [G-14C]nepetadiol (9) were injected into the plant stems with a microsyringe.

**Measurement of Radioactivity.** Radioactivity of the samples were measured by adding quadruplicate aliquots to scintillation vials containing 10 ml of toluene-ethanol scintillation solution (10) and counting in a Model 3320 Packard TriCarb scintillation spectrometer.

**Thin-Layer Chromatography.** The reaction mixtures, essential oils, and preparative GC fractions were analyzed by use of TLC on silica gel plates, both analytical (5 × 20 × 0.01 cm) and preparative (20 × 20 × 0.2 cm). The developing solvents used were hexane:acetone:ethanol (40:10:4, v/v/v). The bands were scraped from the plates and the fractions eluted with ethyl acetate and dried under nitrogen.

**Preparative Gas-Liquid Chromatography.** Preparative GC was performed on a Varian Aerograph Series 700 GLC unit equipped with a hydrogen flame ionization detector, a variable stream splitter, and an automatic collection apparatus. The column used was a 16-foot × ½-inch silanized glass column packed with 25% Apiezon L on Anachrom ABS and operated at 180-240 C. Compounds were trapped in special spiral-path traps cooled in acetone/dry ice.

**Analytical Gas-Liquid Chromatography.** Analytical GC was performed on a modified Barber-Coleman Model 5000 gas chromatograph (11) equipped with a hydrogen flame ionization detector. The columns used were ¼ inch silanized glass tubes packed as above, 8 to 12 feet in length. The column oven was kept at 160 C, the injector at 230 C, and the detector at 275 C.

**Gas Chromatography/Mass Spectrometry.** Low-resolution mass spectra were obtained on a prototype (11,12) of the LKB-9000 combination GC/MS instrument constructed in the laboratory of Dr. Ragnar Ryhage (Karolinska Institutet, Stockholm, Sweden). Spectra were obtained as compounds appeared from the gas chromatograph using a 25% Apiezon L column on Anakrom ABS under the following conditions: 20 eV or 70 eV ionization voltage, 3.5 kV accelerating voltage, 20 µA trap current, 1.7 kV electron multiplier voltage, 310 C source temperature, 250 C separator temperature, 20-30 ml/min
helium flow rate, and 160 C column temperature. A record of the total ionization current obtained from the collector plate in the analyzer served as the gas chromatographic tracing.

**High-Resolution Mass Spectrometry.** High-resolution mass spectrometric analysis was conducted with a du Pont CEC-21110C double-focusing mass spectrometer equipped with a photoplate. Peak matching against perfluorokerosene was used for accurate mass determination.

**RESULTS**

**Metabolism of Nepetalactones (1 and 2).** A mixture of [G-14C]-4α,7α,7αα- and [G-14C]-4α,7α,7αβ-nepetalactone (1 and 2, respectively; about 90:10) was prepared as described in the Experimental section. An 8.3-mg portion of this mixture with radioactivity 14.9 × 10^5 dpm was administered with a microsyringe to a plant that weighed approximately 200 g, which was then kept in a closed chamber for 3 days (8,9,13) and worked up as previously described. The recovered radioactivity was: 10.4 × 10^5 dpm as CO2, 0.025 × 10^5 dpm in the essential oil fraction, and 3.63 × 10^5 dpm in the residue remaining after distillation, for total recovery of approximately 96%. There was no significant carbon-14 in the water remaining from the steam distillate. The dried solid residue was subjected to the following treatments: hexane extraction (three times), which gave an extract with 1.97 × 10^5 dpm, then ethanol extraction (three times), which gave an extract with 0.35 × 10^5 dpm; the remaining residue contained 1.29 × 10^5 dpm. The hexane was evaporated at room temperature under a gentle stream of nitrogen and the residual products were separated by TLC. An unresolved mixture of 4 and 5 gave the dominant peak (86% of radioactivity administered); the TLC showed three additional minor compounds and a spot at the origin but these were not studied further, nor was the ethanol extract or the remaining solid residue.

The average amount of dihydronepetalactones found in the steam-volatile fraction was about 0.002 mg/g fresh weight of the *N. cataria* plants. This was in contrast to nepetalactones, the major organic components of the steam distillate, which contributed about 1-2 mg/g fresh weight. Since the steam distillate from a plant fed with [G-14C]-nepetalactones contained only about 1-2% of the total radioactivity recovered, it was not further studied.

**Metabolism of 4α,7α,7αα- and 4α,7α,7αβ-Dihydronepetalactones.** A mixture of [G-14C]-4 and [G-14C]-5 was administered to catnip plants (14 mg/plant), the specific activity fed varying from 7.9 × 10^3 dpm/mg to 28.1 × 10^3 dpm/mg; the plants were allowed to grow for 14-24 h.

After steam distillation of the essential oil from plants that had been treated, the residue was hydrolyzed in 2N HCl as described in the Materials and Methods section and then subjected to steam distillation a second time. Both essential oils were analyzed. Figure 2 shows the GC tracing for the essential oil before hydrolysis (essential oil I) and that following hydrolysis (essential oil II). As shown previously (13), 1 (rel. R_t = 1.00) is by far the major component of essential oil I, but this also contains components of essential oil II. Essential oil of unmodified *N. cataria* plants yielded a small peak for 4, con-
firming that this isomer is the naturally occurring one in *N. cataria*. Essential oil I was divided into four fractions by preparative GC. Table I showed the identities of these fractions established by mass spectral characteristics and comparison of relative time with those of authentic samples.

Unknown 1, a methylocyclopentane monoterpenoid of molecular weight 166, was assigned the structure of \( \Delta^4 \)- or \( \Delta^{4(7a)} \)-nepetalactol (6) on the basis of its mass spectral fragmentation pattern (2,4,13). The mass spectra of Unknowns 1 and 2 are shown in Fig. 3 along with the proposed structures. The spectrum of Unknown 1 resembles that of methylocyclopentane monoterpenoids but there are a few key exceptions. The \((M+1)/(M+2)\) ratio for \( M^+ \) at \( m/z \) 166 fits the molecular formula \( C_{10}H_{14}O_2 \). The molecular ion undergoes loss of water to yield an ion at \( m/z \) 148, a dehydration confirmed by a metastable ion at \( m/z \) 132.0. The series of odd-electron ions at low abundances at \( m/z \) 67, \( m/z \) 69, \( m/z \) 81, \( m/z \) 95, \( m/z \) 109, and \( m/z \) 123 in the spectrum of Unknown 1 is typical of the methylocyclopentane moiety found in nepetalactones, but one more double bond is present so there is also a series at \( m/z \) 65 and \( m/z \) 67, \( m/z \) 79, \( m/z \) 93, \( m/z \)

![Figure 3. Mass spectra and proposed structures for Unknown Compounds 1 (6) and 2 (7).](image)

**TABLE 1. Components of the preparative gas chromatography fractions from metabolism of 1 and 4**

<table>
<thead>
<tr>
<th>Component (code)</th>
<th>Total, dpm</th>
<th>Radioactivity, dpm/mg</th>
<th>( R_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Hexen-1-ol (9)*</td>
<td>1,005</td>
<td>505</td>
<td>0.15</td>
</tr>
<tr>
<td>4( \alpha ),7( \alpha ),7( \alpha \alpha )-Nepetalactone (1)</td>
<td>210</td>
<td>18</td>
<td>1.00</td>
</tr>
<tr>
<td>4( \alpha ),7( \alpha ),7( \alpha \beta )-Nepetalactone (2) and probably 4( \alpha ),7( \alpha ),7( \alpha \beta )-nepetalactone (3)(^b)</td>
<td>396</td>
<td>8,351</td>
<td>1.15</td>
</tr>
<tr>
<td>( \Delta^4 )- or ( \Delta^{4(7a)} )-Nepetalactol (6)</td>
<td>1,320</td>
<td>11,321</td>
<td>1.33</td>
</tr>
<tr>
<td>4( \alpha ),7( \alpha ),7( \alpha \alpha )-Dihydronepetalactone (4) and 4( \alpha ),7( \alpha ),7( \alpha \beta )-Dihydronepetalactone (5)</td>
<td>3,315</td>
<td>19,650</td>
<td>1.70</td>
</tr>
</tbody>
</table>

* Identified by comparison of mass spectral data with those for authentic cis- and trans-3-hexen-1-ol supplied by Western Utilization Research Laboratory of the U. S. Department of Agriculture, Albany, CA.

* 2 and 3 could be completely resolved by preparative GC so the radioactivity value represents the total label in the compounds.
107 and \( m/z \) 121. Significant peaks at \( m/z \) 77 and \( m/z \) 91 indicate that benzyl and tropylium ions result from loss of neutral hydrogen molecule from the ions at \( m/z \) 79 and \( m/z \) 93, respectively. These transitions are verified by the signals of metastable ions at \( m/z \) 75.1 and \( m/z \) 89.0, respectively. Since the molecular weight is 166, the same as for nepetalactones, the additional double bond in the cyclopentane ring must mean a loss of unsaturation elsewhere. The loss of \( H_2O \) from the molecular ion indicated that the lactone carbonyl group had been reduced to a hydroxyl group and thus converted to a cyclic hemiacetal group. The position of the double bond in the methylocyclopentane ring was not determined but it is probably conjugated with one in the hemiacetal ring. Other fragmentations were like those found in the mass spectra of the nepetalactones, which have been discussed previously (2,4).

The GC analysis (Fig. 2) of essential oil II, obtained by steam distillation of either normal or treated plant residues after 2N HCl hydrolysis, yielded new compound \( \text{7} \), the second unknown, not found in essential oil I. This compound must have been released or formed by the treatment, perhaps via an ester or glycoside hydrolysis. The mass spectrum of \( \text{7} \) shows a molecular ion (\( M^+ \)) at \( m/z \) 154, which is consistent with the \( (M+1)/(M+2) \) ratio; high-resolution mass spectrometry gave the empirical formula \( C_9H_{14}O_2 \). The high-resolution data shown in Table II, the similarity of fragmentation pattern to those of the nepetalactones, and the loss of \( H_2O \) yielding an ion at \( m/z \) 136 indicate that this component is also a cyclic hemiacetal but with one fewer methylene group and one fewer double bond than nepetalactones. The loss of unsaturation is again attributed to the reduction of the lactone carbonyl group to a hydroxyl group and hemiacetal formation. The odd-electron ion series at \( m/z \) 67, \( m/z \) 81, \( m/z \) 95, and \( m/z \) 109 indicates that there is no unsaturation present in the methylocyclopentane moiety. The missing \( CH_2 \) unit was identified as part of a methyl group (C-8) in the numbering scheme shown in Fig. 1. Since the fragmentation pattern resembled that of methylocyclopentane and no peak at \( m/z \) 124 corresponding to loss of two methyl groups, was found, the obviously missing \( CH_2 \) unit came from this methyl group. From these data the structure for \( \text{7} \) and the name de-4-methylnepetalactol shown in Scheme 1 are proposed.

Hydrolysis with 2N HCl released relatively large quantities of \( \text{4, 5, and 7} \) (Table III); these do not normally steam distill readily. Essential oil I was 0.24% of the wet plant weight while that of essential oil II amounted

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**Table II. High resolution mass spectrometric data for de-4-methylnepetalactol (7)***

<table>
<thead>
<tr>
<th>Composition</th>
<th>( m/z )</th>
<th>Nominal</th>
<th>Accurate</th>
<th>( \Delta M^* ) ppm</th>
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<tbody>
<tr>
<td>( C_9H_{14}O_2, M^+ )</td>
<td>154</td>
<td>154.0994</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Fragment ions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_9H_{13}O )</td>
<td>136</td>
<td>136.0936</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>( C_9H_{13} )</td>
<td>109</td>
<td>109.1010</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>( C_9H_{12}O )</td>
<td>109.0653</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_9H_9 )</td>
<td>81</td>
<td>81.0704</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>( C_9H_7 )</td>
<td>67</td>
<td>67.0547</td>
<td>0.1</td>
<td></td>
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</tbody>
</table>

* \( \Delta M \) is the deviation from the nearest perfluorokerosene exact mass.

b \( C_9H_{13} \) peak represents the high-abundance ion of the doublet at \( m/z \) 109.

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**Table III. Major components of essential oil II obtained after HCl hydrolysis of the stripped residue from metabolism of \( \text{4 and 5} \).***

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity, mg</th>
<th>Radioactivity, Total, dpm</th>
<th>Sp. activity, dpm/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>De-4-methylnepetalactol (7)</td>
<td>1.4</td>
<td>1,420</td>
<td>1,000</td>
</tr>
<tr>
<td>4aa,7a,7aa-Dihydronepetalactone (4)</td>
<td>11.4</td>
<td>6,950</td>
<td>610</td>
</tr>
<tr>
<td>4aa,7a,7aa-Dihydronepetalactone (5)</td>
<td>4.1</td>
<td>1,850</td>
<td>450</td>
</tr>
</tbody>
</table>
to 0.92%. Therefore, the 2N HCl hydrolysis released four times as much steam-volatile material as was free in the plants originally, including some components not found in essential oil I. This observation is significant for distillation of essential oils and biosynthetic and biodegradation studies of nepetalactones; these compounds, especially the immediate precursors, are now shown to be present in large part in non-steam-volatile form in the plant. Acid catalysis would promote hydrolysis of a glycosidic linkage in precursors of 4 and 5. 7 may also have been formed or released by acid hydrolysis since its specific activity was similar to that of 4 and 5. The 30 to 40-fold dilution of the specific activities of 4 and 5 indicates that a pool of these compounds in unlabeled form was either present or released by acid hydrolysis.

6, not detectable in plants not treated with 4 and 5, is probably a catabolite of the exogenous dihydroepetalacones. 7, found in essential oil II of both (4 and 5)-treated and nontreated plants, is also likely a catabolite of dihydroepetalacones and/or nepetalactones. The catabolism of [G-14C]nepetalactones to 14CO2 has been demonstrated previously (9,11) and such degradations could proceed through a degenerate monoterpengoid intermediate.

**Metabolism of [G-14C]Nepetadiol.** Two [G-14C]-9 biosynthesis experiments were conducted. In Experiment I, 5.7 mg (15 × 10^4 dpm) of [G-14C]-2 (specific activity 26 × 10^3 dpm/mg) were administered to a twelve-week-old N. cataria plant. The plant was allowed to metabolize for 10 hr, then the plant was analyzed as described previously. In Experiment II, 12.4 mg (15 × 193 dpm/mg) were administered to a sixteen-week-old N. cataria plant. After 18 hr the plant was analyzed as described for Experiment II.

In each case only a small amount of the label was found in the initial steam-volatile crude oils (3.2 × 10^3 dpm in Experiment I and 1.1 × 10^3 dpm in Experiment II). Since 9 is not steam-volatile, any of it that was unreacted would be found in this fraction. The methanol extract of the residue from Experiment I contained 89 × 10^3 and the solid residue 55 × 10^3 dpm; thus 98% of the total radioactive material was recovered. When this methanol-extracted residue was treated with 2N HCl and steam distilled again only a negligible amount of label (560 dpm) was contained in the volatile fraction. In Experiment II the steam-volatile fraction contained 12 × 10^3 dpm while the residue contained 130 × 10^3 dpm — a 94.5% recovery of the administered radioactive label. The lower total recovery of such material may be due to further catabolism of [G-14C]-9 to give 14CO2. The same residue extracted with methanol gave an extract containing 72 × 10^3 dpm and a solid containing 15 × 10^3 dpm; the discrepancy between the total of these and the 130 × 10^3 dpm previously referred to is unaccountable.

The oils from the initial steam distillation yielded purified 1 and 2 with negligible incorporation of label: 0.08% and 0.25% for 1 and 0.03% and 0.25% for 2 as shown in Table IV. These values are only about 25% of the comparable incorporation values for [G-14C]-4 and -5. 6 was not present in the nepetadiol-treated plants.

The methanol extract of the steam distillation residue from the first experiment was shown by TLC to contain 1 and several diastereoisomeric dihydroepetalacones but no 9. Since the essential oil from the HCl-treated plant material had negligible radioactivity, no analysis was done.

In Experiment II, GC analysis of the essential oil from the HCl-treated plant material showed several peaks. Preparative GC produced several diastereoisomeric dihydroepetalacones 4 and 5 and unknown compounds that proved to be 10 and 11. Un-
fortunately, 7 was represented only as a shoulder on the peaks of 10 and 11 on the column, and all gave the same molecular ion, M⁺ 154. The mixture consisted of about 75% 7 and 35% 10 and 11. Their radioactivity amounted to 14 × 10⁷ dpm. The base peak in the mass spectrum of each unknown was at m/z 81. Some similarities were found in the mass spectrum of 7 and those of 10 and 11 but the transitions that differed were more important in structure elucidation. In 10 and 11 the transition from m/z 154 to m/z 123 involved loss of a CH₂OH fragment (M⁺-31), but this was absent from the spectrum of 7. The transition from m/z 93 to m/z 91 was indicated by the spectrum of 7 but not by those of 10 and 11. Other ions had the same nominal mass but the pathways of formation of these ions were different (3). No detailed fragmentation study is described here, but has been reported (10); however, comparison of the three mass spectra indicated that the structures assigned are reasonable. Separation of these compounds by analytical GC was achieved; the specific activity of a mixture of 10 and 11 was 4.5 × 10⁶ dpm/mg. We propose the following structures, which are dehydration products of 9, and probably represent the first metabolites of nepetadiol (shown in Scheme 1).

In Experiment II radioactivity of the methanol extract of the HCl-treated steam distillation residue was 72 × 10⁷ dpm. Analysis of this extract failed to yield any [G-¹⁴C]-2.

DISCUSSION

Components 1 and 2 were oxidized to CO₂ by N. cataria to a significant extent—68% in 3 days; 25% of the radioactive carbon was in the plant residue remaining after distillation, and only 1.8% in the essential oil (13). Redistillation and analysis indicated that the key compounds bound to the plant residue were 4 and 5, which contained 85% of the radioactivity remaining (13).

The specific activity of 7 was 11.3 × 10⁵ dpm/mg, that of 6 1,003 dpm/mg, and that of 3-hexen-1-ol (8) 505 dpm/mg, when they were formed in the metabolism of [G-¹⁴C]-4 and -5 (specific activity 18.4 × 10⁵ dpm/mg) in N. cataria plants. On the other hand, nepetadiol (9) was transformed by N. cataria plants to 10 and/or 11, which could be converted to de-4-methylnepetalactol (7). No radioactivity was then found in 1, 2, 4, 5, or 6. It was concluded that these catabolites are formed in the sequence 6, then 7, and still later (8). The suggested pathway for partial degradation of nepetalactone and nepetadiols in N. cataria is shown in Scheme 1.

The carbon atoms eliminated in forming 7 from 6, and 8 from 7 probably are introduced into the plant's one-carbon metabolic pool and ultimately oxidized to CO₂.

The new compounds observed in this study are of interest both biosynthetically and catabolically and suggest the need of tracer experiments to determine how they originate and how they fit into the metabolic scheme of the plant. These experiments also reveal a new method of obtaining interesting and important methylcyclopentane monoterpenoids, i.e. by steam distillation of extraction of plant material after hydrolysis in 2N HCl. The isolation of major quantities of the compounds 4, 5, 7, and 8 bound in a nonsteam-volatile form is of interest since they may become the essential oil of the plant. The liberation of essential oils by acid hydrolysis presents a new and interesting method for studying the biosynthetic and biodegradative pathways in plants and a possible source of new natural products.

ACKNOWLEDGMENTS

We thank Gary Pollard for technical assist-

<table>
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<tr>
<th>Fraction</th>
<th>Amt., mg</th>
<th>Radioactivity</th>
<th>Sp. activity, dpm/mg</th>
<th>Incorporation, %</th>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>7.1</td>
<td>120</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.8</td>
<td>49</td>
<td>60</td>
</tr>
<tr>
<td>Exp. II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>4.0</td>
<td>248</td>
<td>62</td>
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<tr>
<td>2</td>
<td></td>
<td>0.2</td>
<td>182</td>
<td>828</td>
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TABLE IV. Radioactivity distribution between nepetalactones 1 and 2 in the preparative gas chromatography fractions from [G-¹⁴C]nepetadiol metabolism experiments.
ance, Stuart Scheppele for high-resolution mass spectrometric analyses, O. C. Dermer and E. D. Mitchell for helpful suggestions, and E. J. Eisenbraun for nomenclature of nepetalactones.

REFERENCES