

Isolation and Identification of Nepetalactone Diastereomers from Commercial Samples of *Nepeta cataria* L. (Catnip): An Introductory Organic Laboratory Experiment

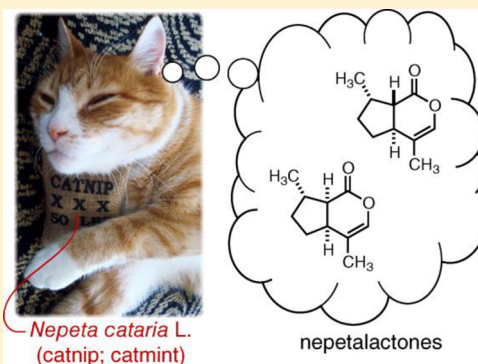
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S Supporting Information

ABSTRACT: The essential oil of *Nepeta cataria* L. (catnip), an herbaceous plant known popularly as a stimulant for the domestic cat, is enriched in two diastereomeric iridoid lactones (nepetalactones) that vary in ratio. Although the diastereomers are chromatographically separable and exhibit different 1D NMR spectra, it is not possible to differentiate between them chromatographically or spectroscopically without previous knowledge or more complex NMR experiments; however, the trans-fused lactone readily epimerizes to the thermodynamically more stable cis-fused lactone upon treatment with base. Students in the introductory organic laboratory establish the presence of the two major nepetalactones in catnip essential oil by TLC and GC–MS analysis, assigning TLC spots and GC peaks to specific lactones by chromatographic comparison of the essential oil with the product of epimerization (i.e., cis-fused lactone) and determining the diastereomeric ratio of nepetalactones by GC peak integration.

KEYWORDS: Second-Year Undergraduate, Hands-On Learning/Manipulatives, Laboratory Instruction, Organic Chemistry, Diastereomers, Gas Chromatography, Mass Spectrometry, Natural Products, NMR Spectroscopy, Thin Layer Chromatography



Nepetalactones are iridoid monoterpenoids found in certain plants of the genus *Nepeta*, consisting of nearly 300 species in the mint family (Lamiaceae).¹ The configuration at C7 is almost invariably (*S*),² and although 7(*S*)-nepetalactones exist as four diastereomers, the oil obtained by steam-distillation of *Nepeta cataria* L. (catnip, catmint) is enriched in the trans-fused (4*aS*,7*S*,7*aS*)-nepetalactone (**1**) and the cis-fused (4*aS*,7*S*,7*aR*)-nepetalactone (**2**) (Scheme 1).³ The diastereomeric ratio changes significantly throughout the growing season and can vary between samples of dried plant material or commercial oil.⁴ Although **1** and **2** are separable by chromatography and exhibit different 1D NMR spectra that are consistent with the general structure of nepetalactone, it is not possible to differentiate between the two diastereomers chromatographically or spectroscopically without previous knowledge or more complex NMR experiments; however, the trans-fused lactone **1** readily epimerizes to the thermodynamically more stable cis-fused lactone **2** upon brief treatment with base (Scheme 1),² affording a quick and straightforward method to distinguish between each diastereomer by either TLC or GC analysis: *the TLC spot or GC peak that diminishes or disappears after epimerization must correspond to trans-fused lactone 1.*

■ BACKGROUND

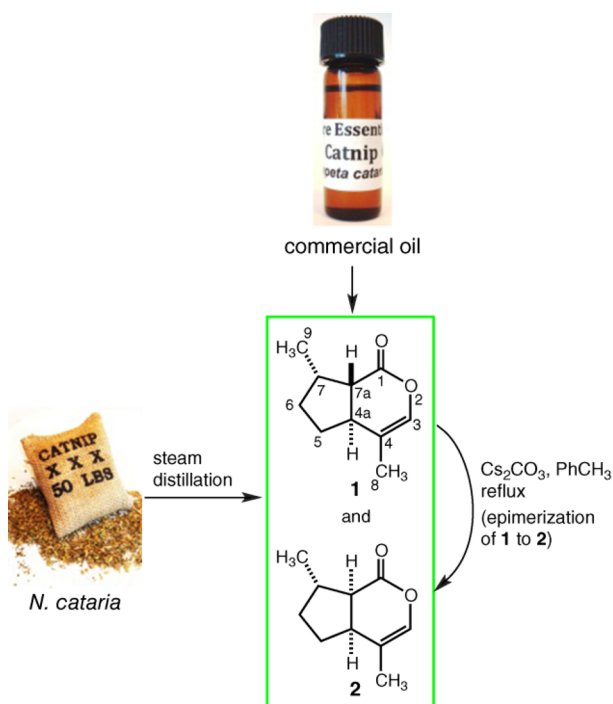
Student interest is often stimulated by natural products possessing biological activity, and undergraduate lab experi-

ments resulting in their isolation have been employed for decades.⁵ The nepetalactones, fused bicyclic enol lactones, are well-known monoterpenoids not often described in organic textbooks. They are the major steam-volatile components of catnip, an herbaceous plant indigenous to Eurasia that is cultivated worldwide for ornamental, commercial, ethnobotanical, and culinary uses⁶ and known widely as a stimulant for the domestic cat and other felids.⁷ Medicinal preparations from the plant have long been used as folk remedies for a variety of human ailments and the oil has been reported to have antimicrobial activity.⁶ Nepetalactones are components of insect pheromone blends² and defense sprays.⁸ They have long been known to repel various insect species⁹ and have recently been reported as a sustainable resource for the preparation of potent nepetalactone-derived insect repellents with levels of repellency rivaling those of DEET (*N,N*-diethyl-3-methylbenzamide), the well-known commercial, petroleum-based repellent.¹⁰

Despite the ready availability of catnip oil, no experiments for the introductory organic lab have been reported involving isolation or identification of diastereomeric lactones **1** and **2**. Fortunately, they are the principal components of catnip oil, and the facile epimerization of **1** to **2** makes them easily distinguishable by equilibration and chromatographic analysis.

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Scheme 1. Epimerization of Nepetalactone Isomers in Catnip Essential Oil



Beyond learning common techniques for isolating an essential oil from its natural source, student goals for this experiment mirror a real-world problem that might be encountered by a natural products chemist: establishing **1** and **2** as the principal components in catnip essential oil and identifying them by a combination of chemical and analytical methods to determine their ratios in a variety of samples. Students accomplish these goals by (i) using analytical techniques (TLC, GC–MS, NMR) to establish the presence of **1** and **2** in catnip oil, (ii) obtaining total energies from Chem3D MM2 minimized structures to calculate the 1:2 ratio anticipated upon equilibration, (iii) epimerizing the lactones with base, (iv) chromatographically comparing the essential oil with epimerized product (i.e., **2**) and assigning TLC spots and GC peaks to **1** and **2** by noting which spot and peak diminished or disappeared upon equilibration, and (v) determining the 1:2 ratio in the essential oil by GC peak integration. Through experimentation, students learn how diastereomers can differ chromatographically and spectroscopically, and yet remain difficult to identify using standard analytical techniques. They learn the value of theoretical calculations in experimental endeavors, and the epimerization they perform also serves as an experimental test of their equilibrium calculation. They also directly compare TLC and GC methods for analyzing mixtures and experience firsthand the benefits of collaboration by sharing data to analyze multiple samples within the allotted lab time.

OVERVIEW OF THE EXPERIMENT AND RESULTS

Students were informed that oil from *N. cataria* is predominantly a mixture of epimerizable nepetalactone diastereomers **1** and **2**. They either obtained catnip essential oil by steam-distillation of commercial dried plant material or were given samples of the commercial essential oil.

Since 2008, 170 students enrolled in a second-semester introductory organic chemistry laboratory course completed this experiment in either two 4-h lab periods or a single 4-h lab period if steam-distillation was omitted and the epimerization and analysis was performed with commercial oil samples. Complete experimental instructions for students, screenshots from Chem3D MM2 energy minimizations of nepetalactone diastereomers, a sample equilibrium constant calculation, and full-scale spectral data are provided in the Supporting Information.

Isolation and Analysis of the Essential Oil

Internal steam-distillation (hydrodistillation) was used to collect distillate from a heated mixture of water and dried catnip obtained from local stores. Approximately 50–90 min was required to collect 90–100 mL of distillate using 5 g of dried plant material. Extraction of the distillate with an organic solvent afforded approximately 10–15 mg of a viscous oil or semisolid upon evaporation.¹¹ During the distillation, for each different plant sample assigned, one student performed a small-scale extraction on an aliquot of distillate followed immediately by capillary GC–MS analysis of the extract. Those students then shared GC–MS data with students analyzing the same plant sample.

Typical GC–MS results are shown in Figure 1 (chromatogram 1) and Table 1 shows student data from a variety of

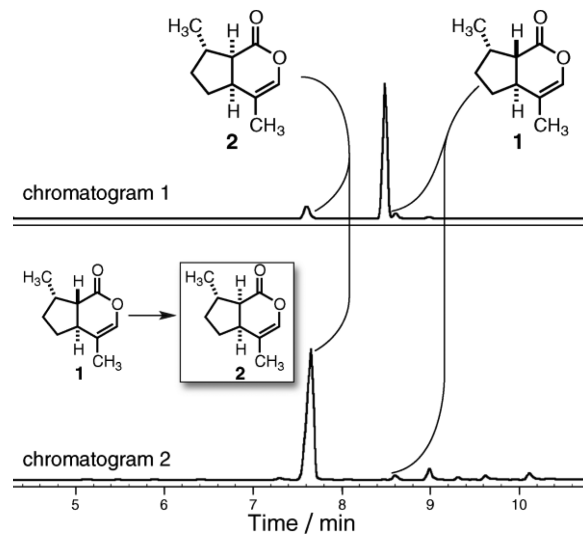


Figure 1. Chromatograms of crude oil from steam-distillation (**1**) and product of epimerization (**2**).

samples. Mass spectra of **1** and **2** display intense molecular ions (m/z 166) and essentially identical fragmentation (Figure 2). Because most oils are predominantly mixtures of **1** and **2**, often with one in great excess, NMR spectra were usually relatively clean (Figure 3).¹³

Epimerization of Nepetalactones and Product Analysis

Students referred to GC–MS and NMR data to answer questions provided to help them establish the presence of nepetalactone diastereomers in catnip oil samples and to conclude that without further information it was not possible to assign TLC spots or GC peaks to a specific diastereomer. Each student dissolved their oil in toluene, added Cs_2CO_3 , and refluxed the suspension for at least 20 min to equilibrate the nepetalactones.¹⁴ For each different commercial catnip sample

Table 1. GC–MS Analysis of Catnip Essential Oils either Isolated by Steam Distillation (StD) or Purchased from a Commercial Source (Oil)

Catnip Sample (Manufacturer; StD or Oil)	Essential Oil Profile ^a	Ratio of 1:2
A (Pet Gold Products, CA; StD)	1 (92%); 2 (4.5%); dihydronepetalactone (1.2%); caryophyllene oxide (1.1%); unidentified nepetalactone isomer (<1%) ^e	95:5
B (Vo-Toys, Inc., NJ; StD)	1 (91%); 2 (4.4%); caryophyllene oxide (2%); dihydronepetalactone (1.3%); unidentified nepetalactone isomer (<1%) ^c	95:5
C (Back Bay Pet Co., ME; StD)	2 (95%); unidentified nepetalactone isomer (1.7%) ^c ; 1 (<1%); caryophyllene oxide (<1%)	1:99
D (Worldwise, Inc., CA; StD)	2 (96%); caryophyllene (1.3%); 1 (<1%); caryophyllene oxide (<1%); unidentified nepetalactone isomer (<1%) ^c	1:99
E (Petco Animal Supplies, CA; StD)	1 (90%); 2 (7%); caryophyllene (1.1%); unidentified nepetalactone isomer (1%) ^c ; caryophyllene oxide (<1%)	92:8
F (Castor & Pollux Pet Works, OR; StD)	caryophyllene (24%); 2 (21%); caryophyllene oxide (10%); unknown components (13%, 11%, 9%, 6%); 1 (2.4%); dihydronepetalactone (1%)	10:90
G (Hartz Mountain Corp, NJ; StD)	menthol (77%); menthone isomers (8.8%); piperitone (5.2%); pulegone (3.3%); menthol acetate (3%); caryophyllene (<1%); eugenol methyl ether (<1%); germacrene D (<1%)	1 and 2 not detectable by GC–MS
H, I, J (Sleeping Dragons Co., NM; Oil) ^b	1 and 2 (70–86%); caryophyllene (1.8–8.6%); caryophyllene oxide (1.5–5%); unidentified nepetalactone isomer (<1%) ^c	H: 43:57 I: 54:46 J: 64:36
K (Appalachian Valley Natural Products, MD; Oil)	1 (47%); 2 (33%); caryophyllene (13%); α -caryophyllene (1.5%); unidentified nepetalactone isomer (2%) ^c ; caryophyllene oxide (1.1%)	59:41

^aTentative identification by MS comparison with the NIST Mass Spectral Database (some minor components could not be identified). ^bSamples purchased from the supplier at intervals over two years. ^cPossibly (4aR,7S,7aS)-nepetalactone.¹²

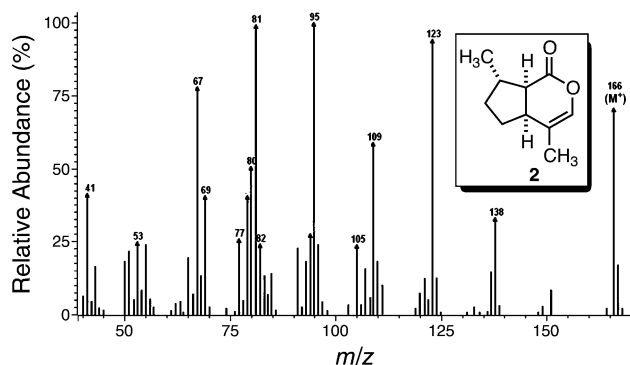


Figure 2. Mass spectrum of cis-fused lactone **2** (identical to spectrum of trans-fused lactone **1**).

assigned, only one student performed GC–MS analysis of their reaction product. They shared the data with students assigned the same commercial sample and all compared it with GC–MS data from the untreated oils. Of the two major peaks that corresponded to nepetalactones in the GC trace of the untreated oil (Figure 1, chromatogram 1), only one either diminished or disappeared in the GC trace of the reaction product (Figure 1, chromatogram 2), inferring that the remaining nepetalactone peak must correspond to **2**.¹⁵ While representative samples were being analyzed by GC–MS, the rest of the class assigned TLC spots to **1** and **2** by directly spotting their reaction mixture versus a sample of their untreated catnip oil (Figure 4).¹⁶

After GC peak assignment, students used peak integral values to obtain the 1:2 ratio for each plant sample analyzed. Table 1 includes results from GC–MS analysis of oils from eleven samples of catnip. Of the seven dried samples (A–G) students analyzed, five (A–E) contained either **1** or **2** as the predominant steam-volatile product along with 1–8% of the other diastereomer. One sample (F) contained a mixture of **1**, **2** (with a 1:2 ratio of 10:90) and significant amounts of several

other substances, which complicated TLC analysis. Surprisingly, one spurious sample (G) contained predominantly menthol with no detectable amounts of nepetalactone; possibly either a nepetalactone-less *Nepeta* variant¹⁷ or a *Mentha* species. All of the commercial essential oil samples students examined (H–K) were from *N. cataria* chemotypes containing significant amounts of both **1** and **2**.

Standard 1D NMR and DEPT experiments permit students to make key C and H shift assignments for **1** and **2**: DEPT-90 and DEPT-135 experiments permit general assignment of methyl carbons (C8 and C9), methine carbons (C3, C4a, C7, C7a) and methylene carbons (C5 and C6); carbon shifts for C1 (C=O), C3 and C4 (sp^2 carbons) are easily assigned, with C3 and C4 distinguishable by DEPT experiments; proton shifts for C3 (vinyl), C8 (methyl singlet) and C9 (methyl doublet) are also readily assigned.

HAZARDS

Eye protection, lab coats, and gloves are required as all materials used in this experiment are irritants or harmful if inhaled or swallowed, including catnip oil and its nepetalactone constituents. Catnip oil, toluene, petroleum ether, hexanes, ethyl acetate, diethyl ether, ethanol, and methanol are flammable. Dichloromethane and chloroform-*d* are inhalation hazards and suspected carcinogens. CS_2CO_3 is moderately toxic by ingestion. Iodine and ethanolic solutions of phosphomolybdic acid are corrosive. Exposure of skin or eyes to UV light should be avoided.

SUMMARY

All students who performed the steam-distillation obtained essential oil; for example, in 2010, 47 students in three lab sections obtained an average yield of 13.9 ± 6.6 mg from 5 g of plant material. Nepetalactones were easily identified by GC–MS due to their prominent M^+ and $[M - CH_3]^+$ peaks. With only a basic knowledge of NMR spectroscopy, most students, with few exceptions, readily assigned key nepetalactone ¹H and

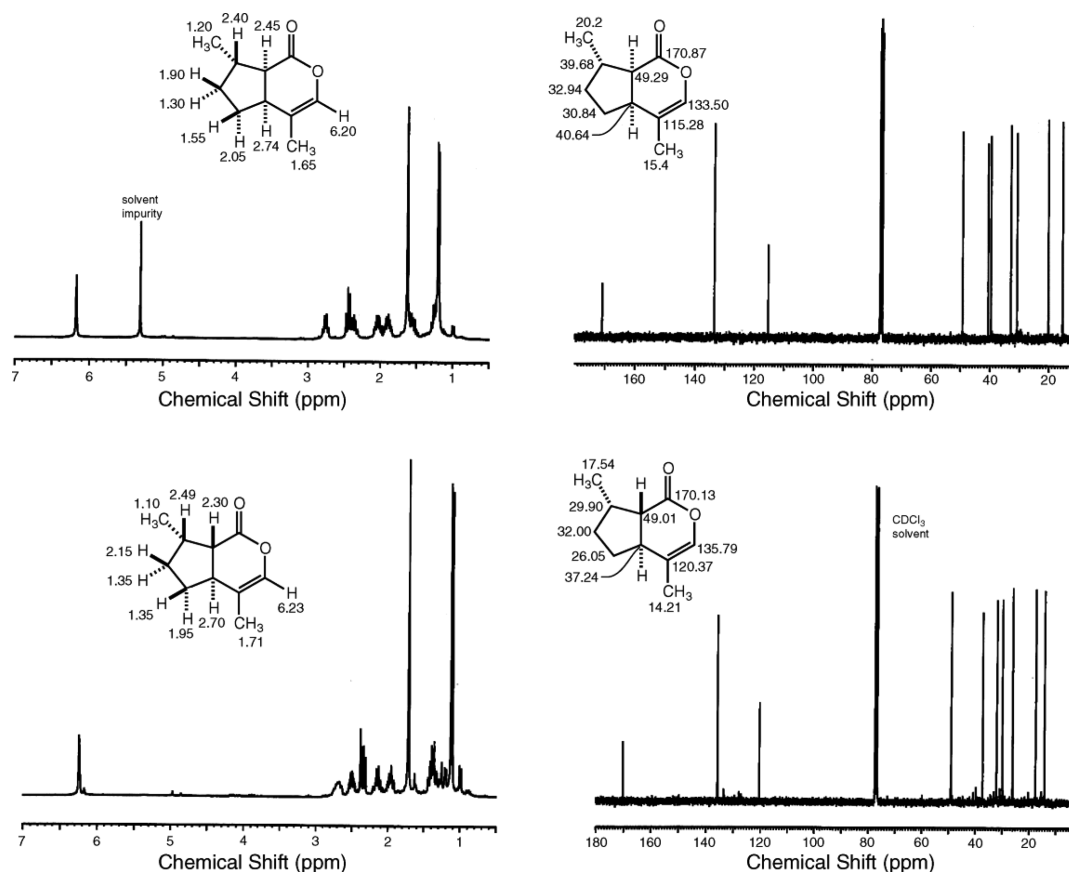


Figure 3. ^1H and ^{13}C NMR spectra of crude oils isolated by students from steam-distillation of commercial samples C (cis-fused isomer) and A (trans-fused isomer) (Table 1); peak assignments are from ref 2.

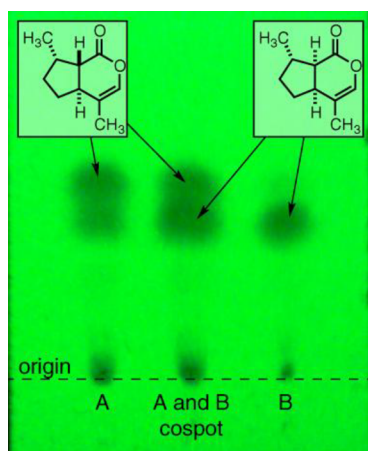


Figure 4. UV-visualized TLC plate of commercial catnip oil before (A) and after (B) epimerization.

^{13}C peaks, and postlab questions helped students to conclude that **1** and **2** are not distinguishable by 1D NMR alone. Epimerization is rapid and the outcome was in agreement with student calculations from MM2 data. Co-spotting samples made TLC analyses straightforward for most students despite the similar R_f values of **1** and **2**, and no one failed to assign GC peaks to specific diastereomers due to the often dramatic difference in traces before and after epimerization (Figure 1); consequently, students readily established the 1:2 ratio in each sample by GC peak integration (Table 1). Providing commercial oil samples permitted students to focus more on

nepetalactone reactivity and analytical aspects, greatly shortening the experiment without significantly affecting its pedagogical value. This experiment can be easily tailored to fit a variety of curricular needs; for example, it could be limited to TLC co-spotting of commercial oil samples versus epimerization products to correlate TLC spots with specific nepetalactone diastereomers, or extended to include complete C and H chemical shift assignments by a series of COSY and NOESY NMR experiments.²

■ ASSOCIATED CONTENT

📄 Supporting Information

Experimental handouts for students; notes for instructors; capillary GC-MS, ^1H and ^{13}C data for student samples and commercial oils. This material is available via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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- (11) Approximately 80–100 min was required to collect 100 mL of distillate using 10 g of dried plant material, affording 20–40 mg of oil after extraction.
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- (13) Combining oil samples from the same plant source allowed students to obtain carbon spectra relatively quickly using a high-field FT-NMR spectrometer. Alternatively, copies of representative spectra (^1H , ^{13}C , DEPT-90, DEPT-135) obtained in advance could be distributed to the class.
- (14) K_2CO_3 worked equally well but required larger quantities and longer reaction times (≥ 40 min), perhaps due to its low solubility in toluene. To obtain enough material for rapid ^{13}C NMR analysis, epimerization can be performed on a larger scale (200 mg oil; 40 mg Cs_2CO_3 [~ 0.1 equiv]; < 30 min). For a review on use of Cs_2CO_3 in organic synthesis, see Flessner, T.; Doye, S. *J. Prakt. Chem.* **1999**, *341*, 186–190.
- (15) ^1H and ^{13}C NMR spectra for the reaction product are consistent with data for cis-fused lactone **2** reported in refs 2 and 3.
- (16) **1**, R_f 0.33; **2**, R_f 0.27; SiO_2 plates with 254 nm fluorescent indicator, 20:1 pet. ether/EtOAc as eluent, visualization with UV, I_2 and phosphomolybdic acid 20 wt % in EtOH.
- (17) Two main chemotypes are apparent: one with nepetalactones as the predominant oil components and another with citral derivatives predominating; see ref 6.