Isolation and Structure Elucidation of the Terpene β-Thujone from Cedar Leaf Oil

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ABSTRACT: Western red cedar leaf affords an essential oil characterized by high thujone content. Students in an advanced organic chemistry laboratory course isolate a single thujone diastereoisomer from commercially available cedar leaf oil. Treatment of crude oil, containing roughly 70% thujone, predominately as α-thujone (6.5:1), with ethanolic sodium hydroxide leads to epimerization at the chiral α-carbon. Equilibration thus inverts the diastereoisomeric ratio in favor (roughly 2:1) of the β-thujone epimer. Selective formation of the bisulfito addition product from the β-isomer allows for a simple purification via filtration. Release of the free ketone upon treatment with Na2CO3 yields highly pure β-thujone, which is characterized and identified using IR, MS, and NMR spectroscopy.

KEYWORDS: Upper-Division Undergraduate, Laboratory Instruction, Organic Chemistry, Hands-On Learning/Manipulatives, Bioorganic Chemistry, Enantiomers, IR Spectroscopy, Mass Spectrometry, NMR Spectroscopy, Natural Products

Steam distillation of Western red cedar leaf affords an essential oil characterized by high thujone content. In this laboratory project, students in an advanced organic chemistry laboratory course isolate a single thujone diastereoisomer from commercially available cedar leaf oil. Treatment of crude oil, containing roughly 70% thujone, predominately as α-thujone (6.5:1), with ethanolic sodium hydroxide leads to epimerization at the chiral α-carbon. Equilibration thus inverts the diastereoisomeric ratio in favor of the β-thujone epimer (roughly 2:1). Selective formation of the bisulfito addition product from the β-isomer allows for a simple purification via filtration. Subsequent release of the free ketone upon treatment with Na2CO3 yields highly pure β-thujone, which is characterized and identified using IR, MS, and NMR spectroscopy. β-Thujone thus obtained yields a highly instructive set of 1D and 2D NMR spectra that provide opportunities to consider several unique features including the anisotropic shielding of cyclopropyl protons, the negative chemical shift of the cyclopropyl methylene endo proton, diastereotypic methyl groups, and long-range “W” type couplings in a rigid bicyclic system. Relative stereochemical configuration can be established via careful consideration of coupling constants. The thujone unknown, once identified, offers the opportunity to engage students with the story, science, and folklore surrounding the notorious spirit absinthe, the “Green Fairy”.

BACKGROUND

Thujone was once believed to possess a range of central nervous system activities accounting for distinctive mind-altering experiences reported by those indulging in absinthe. Despite past lore and recent cachet, the liquor absinthe has not been demonstrated to contain compounds with unique psychotropic properties. Recent science and current medical thinking suggest absinthism is an apocryphal ailment; its purported symptomatology indistinguishable from that associated with chronic alcohol abuse.1 Dubbed the “Green Fairy”, for the chlorophyll imparted hue obtained during post distillation coloring with herbal material, absinthe is distilled from a mixture composed primarily of wormwood, anise, hyssop, and fennel. Wormwood oil is rich in thujone.

Prohibition of absinthe sales in the United States, France, and most of Europe by 1915 was in part a reaction to the mythology surrounding purported psychotropic properties of this spirit. These legal proscriptions only added to its allure. The simple bicyclo[3.1.0]hexanone derivative, thujone, was suggested to be the constituent responsible for a range of effects including hallucinatory delirium, mania, and seizures. A communication in Nature2 presented no data, but pointed to a structural similarity between thujone (particularly as its enol tautomer) and the cannabinoid, tetrahydrocannabinol (also called THC and the active ingredient in marijuana). It posited that absinthism and marijuana intoxication might derive from activity of these compounds at a common receptor. Later work, informed by the discovery of the cannabinoid receptors CB1 and CB2, found low binding constants and no functional activity for thujone at CB receptors.3 β-Thujone does possess modest stimulant and convulsant activity deriving from GABA receptor modulation4 and slight serotonergic 5-HT3 antagonism.5 Recent analytical work5,6 on vintage preban and modern (now legal) absinthes reveal thujone concentrations well below the threshold of significant biological activity.

Students obtain stereochemically pure (1S,4S,5R)-(−)-thujone 2 (isothujone, β-thujone) from inexpensive, commercially available cedar leaf oil. Treatment of crude oil, containing roughly 70% thujone, predominately as α-thujone (6.5:1), with ethanolic sodium hydroxide leads to epimerization at the chiral α-carbon. Equilibration thus inverts the diastereoisomeric ratio in favor of β-thujone (roughly 2:1). Selective formation of the bisulfito addition product from the β-isomer allows for a simple purification via filtration. Release of the free ketone upon treatment with Na2CO3 yields highly pure β-thujone, which is characterized and identified using IR, MS, and NMR spectroscopy.

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available essential oil produced from the “slash waste” generated during harvesting of western red cedar (Scheme 1). Unlike the wormwood species, Artemisia pontica and Artemisia absinthium, in which the β-diastereoisomer predominates, cedar leaf oil contains predominately α-thujone. However, base promoted epimerization leads to an equilibrated mixture of thujone isomers in which the β-isomer predominates. Only the β-isomer affords a bisulfite addition product upon treatment with aqueous NaHSO3, a result consistent with the accessibility of the exo carbonyl face in this isomer. Filtration of this adduct, and subsequent treatment with aqueous Na2CO3, returns a highly pure ketone for identification. This isolation is a modification of a published procedure1 differing only in the milder, nonacidic conditions for ketone liberation. Recently, laboratory protocols for α-thujone isolation from sage and β-thujone from wormwood appeared in Berger and Sicker’s excellent text.2 Unfortunately, these protocols rely on tedious spinning band fractional distillation and subsequent chromatography and are not viable in a typical undergraduate lab setting.

**EXPERIMENTAL DETAILS**

**Spectroscopic Analysis**

Western red cedar leaf oil (Thuja plicata) was obtained from Citrus and Allied Essences Ltd. (Belcamp MD). The total thujone content of the oil is represented to be approximately 70%. Students are not informed of the provenance of the oil. They are told that the compound of interest is an epimerizable ketone. Capillary GC–MS analysis (see the Supporting Information) was conducted by the students. The thujone isomers are well resolved with α-thujone eluting at 11.82 min and β-thujone at 11.99 min. There are subtle differences in the mass spectra of the two isomers with the most obvious difference in base peaks (m/e 81 for α- and 110 for β-isomer). Integration (uncalibrated) reveals an approximate total thujone content over 80% and an α/β ratio of 6.5:1. 1H NMR of the crude oil (Jeol Eclipse 300 MHz, CDCl3) is consistent with a mixture comprised primarily of α-thujone (see the Supporting Information). A comparison of integrations of the respective H6 endo proton resonances is again consistent with a 6.5:1 ratio. Armed with the GC–MS and 1H NMR data for the raw oil and a full set of 1D and 2D NMR, IR, and GC–MS data for the isolated pure terpene (obtained by the students; see the Supporting Information), the students are asked to determine the structure of each isomer.

**Isolation**

To 7.00 g of essential oil in a 50 mL Erlenmeyer flask is added 5% NaOH in 95% ethanol (5 mL). The mixture is stirred vigorously at room temperature for 30 min. Then, freshly prepared saturated NaHSO3 (10 mL) is added. The mixture is allowed to stir at room temperature until the following lab period. The precipitated bisulfite addition product is collected by suction filtration, pressing and washing with a couple of 2 mL portions of cold 95% ethanol to remove clinging oil. The solid is transferred to a 125 mL Erlenmeyer flask and 10% aqueous Na2CO3 (50 mL) is added. The solution is stirred for 10–15 min at room temperature during which time free ketone separates as an oil. The oil is extracted into ether (3 × 50 mL) and dried over sodium sulfate. Solvent is stripped by rotary evaporation to yield highly pure β-thujone. Students typically recover 2–3.5 g of material. Students characterize the product by GC–MS, IR, and NMR.

**HAZARDS**

Cedar leaf oil may cause eye and skin irritation. Ethanolic sodium hydroxide is caustic. Ether is a highly volatile and flammable solvent. Deliberate ingestion of large quantities of thujone can result in seizures as has been reported in the medical literature.3

**SPECTRAL ANALYSIS AND ROADMAP TO STRUCTURE ELUCIDATION**

The structure of β-thujone is of modest complexity and presents a suitably challenging unknown for an upper-level undergraduate organic chemistry student. Thujone (Figure 1) incorporates interesting functionality with a cyclopropyl group homoconjugated to a cyclopentanone carbonyl and three contiguous stereocenters. One approach to structure elucidation follows. No data relevant to
assignment of absolute configuration is collected; thus, students arrive at one set of enantiomers consistent with all data.

Consideration of the diagnostic regions of the IR confirms the presence of a carbonyl group at 1740 cm\(^{-1}\). Absorption >3000 cm\(^{-1}\) will initially be misleading as students will interpret this as sp\(^2\) C–H stretching.

Working with the molecular ion at 152 amu, a carbon count of 10 from the \(^{13}\)C NMR data and a hydrogen count of 16 evidenced by the integration of the \(^1\)H NMR provide the molecular formula \(C_{10}H_{16}O\). Calculating the index of hydrogen deficiency reveals two unsaturations in addition to the carbonyl. Despite IR absorption in a region typically associated with sp\(^2\) C–H stretching, no proton or carbon chemical shifts appear consistent with vinyl H or C. Lacking evidence supporting the presence of additional carbonyl groups or alkyne functionality, a conclusion is reached that two rings are incorporated. The presence of one isopropyl and one additional methyl group (\(^1\)H NMR and DEPT) forces the student to conceive of a bicylic structure constructed from only six carbons: possibilities are 4,4-fused, 5,3-fused, and [3.1.1]-bridged.

The extremely high-field proton resonances, including the unusual negative chemical shift, suggest the presence of the cyclopropane with ring anisotropy responsible for the extreme shielding. The 6-endo proton, characterized by a chemical shift of \(-0.05\), lies also within the anisotropic shielding cone of the transannular carbonyl in the boat-like conformation. Even low-level modeling software can be used to show that this proton is closer to the carbonyl in the \(\beta\)-isomer and is shifted 0.16 ppm upfield from the corresponding resonance in \(\alpha\)-thujone. Consideration of these features, among others, should lead to the proper conclusion that the system is 5,3-fused. The paradoxical IR data can now be reconciled with the additional \(\gamma\) character devoted to the cyclopropyl C–H bonds with greater \(\pi\) contribution to the strained C–C bonds of the small ring.

Resonances for three hydrogens in the chemical-shift region consistent with positioning \(\alpha\) to the ketone in conjunction with DQF-COSY coupling of one of these to a methyl group establishes with certainty the complete connectivity with the exception of the placement of the isopropyl group. This could be at C1 or C5. Recourse to the DQF-COSY provides an easy means of establishing the links between coupled H's and allows the isopropyl group's position to be fixed at C1 as C5 bears an H coupled to that at C4. Several pieces of information point to a trans disposition for the C4 methyl and C1 isopropyl groups. With the trans arrangement in \(\beta\)-thujone, the exo H4 is 0.3 ppm more deshielded than the endo H4 of the \(\alpha\)-cis isomer as it is not in the anisotropic shielding region of the cyclopropane. Further, it is strongly coupled to the bridgehead H5, whereas the endo H4 of the isomeric cis isomer is not (see comparison of dihedral angles in Supporting Information).

## ASSOCIATED CONTENT

### Supporting Information
Capillary GC–MS analysis of Western red cedar leaf oil; \(^1\)H NMR data for the raw oil; a full set of 1D and 2D NMR, IR, and GC–MS data for the isolated pure terpene. This material is available via the Internet at http://pubs.acs.org.

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## REFERENCES