# α- and β-Thujones (Herbal Medicines and Food Additives): Synthesis and Analysis of Hydroxy and Dehydro Metabolites

Nilantha S. Sirisoma, Karin M. Höld, and John E. Casida*

Environmental Chemistry and Toxicology Laboratory, Department of Environmental Science, Policy and Management, 115 Wellman Hall, University of California, Berkeley, California 94720-3112

Essential oils containing α- and β-thujones are important herbal medicines and food additives. The thujone diastereomers are rapidly metabolized convulsants acting as noncompetitive blockers of the γ-aminobutyric acid-gated chloride channel. Synthesis and analysis of the metabolites are essential steps in understanding their health effects. Oxidation of α- and β-thujones as their 2,3-enolates with oxodiperoxymolybdenum(pyridine)(hexamethylphosphoric triamide) gave the corresponding (2*R*)-2-hydroxythujones assigned by $^{1}$H and $^{13}$C NMR and X-ray crystallography. α-Thujone was converted to 4-hydroxy-α- and -β-thujones via the 3,4-enol acetate on oxidation with peracid and osmium tetroxide, respectively. Ozonation provided 7-hydroxy-α- and -β-thujones, and by dehydration provided the 7,8-dehydro compounds. 4,10-Dehydrothujone was prepared from sabinene via sabinol. The hydroxy and dehydro derivatives are readily identified and analyzed by GC/MS as the parent compounds and trimethylsilyl and methyloxime derivatives. A separate study established that all of these compounds are metabolites of α- and β-thujones.

**Keywords:** *α- and β-Thujones; oxidation reactions; metabolite synthesis; metabolite derivatization; GC/MS analysis*

## INTRODUCTION

α-Thujone (**1**α) and β-thujone (**1**β) are bicyclic monoterpenes which differ in the stereochemistry of the C-4 methyl group (*1*) (Figure 1). The isomer ratio depends on the plant source, with high content of α-thujone in cedarleaf oil (*2*) and β-thujone in wormwood oil (*3*). They are also common constituents in herbal medicines, essential oils, foods, flavorings, and beverages (*2*, *4*, *5*). α-Thujone is perhaps best known as the active ingredient of the alcoholic beverage absinthe which was a very popular European drink in the 1800s and gained considerable notoriety as a preferred liqueur of artists and writers (*3*, *6*−*10*). Although absinthe became an epidemic health problem, leading to a widespread ban in the early 1900s (*8*), its use continues on a small scale either legally or illicitly (*11*). The primary health concern is from consumption in herbal medicines, foods, and drinks, leading to the U.S. National Toxicology Program recommending chronic toxicity studies on thujone in rats and mice (*4*).

Understanding the health effects of the thujone diastereomers requires defining their mode of action and metabolic fate. The acute toxicity of α-thujone can be attributed to blocking the γ-aminobutyric acid (GABA)-gated chloride channel (*3*). Metabolite synthesis and analysis are essential steps in defining the metabolic pathways of α-thujone and β-thujone and the biological activities of their metabolites. Our recent studies of the fate of α-thujone and β-thujone in vitro and in vivo led to identification of the hydroxythujones and dehydrothujones shown in Figure 1 (*3*, *12*). A large number of isomeric hydroxythujones are involved with similar GC characteristics and MS fragmentations. An important step was to establish that all of the hydroxythujones and dehydrothujones can be separated by GC either underivatized or as their trimethylsilyl (TMS) or methyloxime (MOX) derivatives. Synthesis and full chromatographic and spectral characterization of these candidate metabolites and their TMS and MOX derivatives described here makes it possible to identify them and ultimately to determine their health effects.

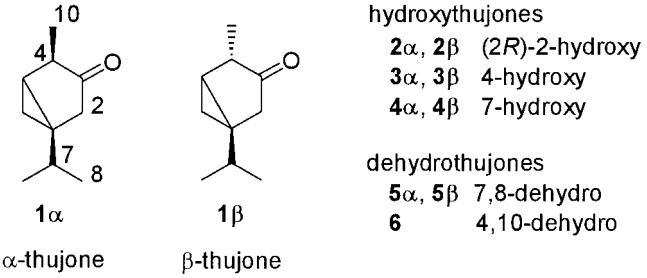



**Figure 1.** Structures of α- and β-thujones and designations for isomeric hydroxythujones and dehydrothujones reported here and identified as metabolites.

## MATERIALS AND METHODS

**Safety.** The preparation of MoOPH involves hydrogen peroxide and should be performed behind a safety shield to minimize explosion hazards (*13*).

**Chemicals.** α-Thujone (99% pure) was from Fluka (St. Louis, MO) and sabinene (70% pure, from nutmeg essential oil) was from R. C. Treatt and Co., Ltd. (Bury St. Edmunds, Suffolk, U.K.). β-Thujone was isolated from wormwood oil (3.2% α-thujone and 35% β-thujone) (*3*) (Lhasa Karnak, Berkeley, CA) by chromatography of a 5-g sample on a silica gel column using 1−5% ethyl acetate in hexane. The β-thujone fractions contained an alkene impurity which was removed by treatment with *m*-chloroperoxybenzoic acid (*m*CPBA) in $CHCl_3$, and chromatography using 3−5% ethyl acetate in

* To whom correspondence should be addressed. Tel: (510) 642-5424. Fax: (510) 642-6497. E-mail: ectl@nature.berkeley.edu.

10.1021/jf001445+ CCC: $20.00 
Published on Web 03/30/2001

**Table 1. $^1$H NMR (300 MHz) Data [$\delta$, $J$(Hz)] in $CDCl_3$**

| Hydrogen | | **1**α[a] | | **1**$\beta$[a] | | **2**α | | **2**$\beta$ | | **3**α[a] |
|---|---|---|---|---|---|---|---|---|---|---|
| 2α | 2.52 | ddd (1.1, 2.6, 18.5) | 2.52 | dt (2.6, 18.5) | 2.54 | br, s | 3.74 | br, s | 2.69 | dd (2.6, 17.9) |
| 2$\beta$ | 2.04 | d (19.0) | 2.10 | d (17.9) | — | | — | | 2.22 | d (17.9) |
| 4 | 2.19 | dq (1.1,7.7) | 2.69 | m | 2.33 | q (7.7) | 2.97 | m | — | |
| 5 | 1.06[b] | dd (4.1, 8.2) | 1.42 | m | 1.15 | m | 1.68 | m | 1.47 | dd (4.1, 8.2) |
| 6α | 0.73 | ddd (2.0, 5.6, 7.7) | 0.57 | m | 0.69 | ddd (2.0,6.2,8.2) | 0.68 | ddd (2.0, 6.7, 7.9) | 0.75 | ddd (2.6, 6.2, 8.2) |
| 6$\beta$ | 0.09 | dd (4.1, 5.6) | −0.06 | dd (5.6,4.1) | 0.31 | dd (4.1,6.2) | −0.32 | dd (4.1, 6.7) | 0.34 | dd (4.1, 6.2) |
| 7 | 1.33 | m (6.7) | 1.42 | m | 1.49 | m (7.2) | 2.32 | m | 1.41 | m (7.2) |
| 8 | 0.92 | d (6.7) | 1.01 | d (6.7) | 1.07 | d (6.7) | 1.04 | d (6.7) | 0.97 | d (7.2) |
| 9 | 0.98 | d (6.7) | 0.98 | d (6.7) | 1.06 | d (7.1) | 1.03 | d (6.7) | 1.03 | d (7.2) |
| 10 | 1.13 | d (7.7) | 0.90 | d (6.7) | 1.18 | d (7.7) | 0.74 | d (7.2) | 1.38 | s |
| OH[c] | — | | — | | 4.61 | br, s | 2.84 | br, s | 2.44 | br, s |

| Hydrogen | | **3**$\beta$ | | **4**α | | **4**$\beta$ | | **5**α | | **6** |
|---|---|---|---|---|---|---|---|---|---|---|
| 2α | 2.78 | dd (3.1, 18.5) | 2.76 | d, m (18.5) | 2.82 | dt (2.6, 18.0) | 2.81 | d, m (18.5) | 2.47 | dd (2.6, 19.5) |
| 2$\beta$ | 2.13 | d (18.5) | 2.16 | d (18.9) | 2.22 | d (18.5) | 2.29 | d (19.0) | 2.27 | d (19.5) |
| 4 | — | | 2.27 | q (7.6) | 2.73 | m | 2.29 | q (7.2) | — | |
| 5 | 1.45 | dd (4.1, 8.7) | 1.33 | dd (4.1, 8.7) | 1.71 | m | 1.47 | dd (4.1, 8.2) | 2.02 | dd (3.6, 8.2) |
| 6α | 0.79 | ddd (3.1, 6.2, 8.7) | 1.10 | ddd (2.6, 5.6, 8.2) | 0.94 | m | 1.17 | m | 1.03 | ddd (2.6,5.1,8.2) |
| 6$\beta$ | −0.02 | dd (4.1, 6.2) | 0.08 | dd (4.1, 5.6) | −0.03 | dd (4.5, 6.2) | 0.34 | m | 0.40 | dd (3.1, 4.6) |
| 7 | 1.47 | m (6.7) | — | | — | | — | | 1.45 | m (6.7) |
| 8 | 1.02 | d (6.7) | 1.19 | s | 1.20 | s | 4.82 | d, m (18.5) | 0.96 | d (6.7) |
| 9 | 0.93 | d (6.7) | 1.29 | s | 1.31 | s | 1.71 | s | 0.91 | d (6.7) |
| 10 | 1.27 | s | 1.15 | d (7.2) | 1.06 | d (7.2) | 1.19 | d (7.2) | 5.7 | s |
| | | | | | | | | | 5.2 | s |
| OH[c] | 2.04 | br, s | 1.82 | br, s | | | — | | | — |

[a] Assignment based on Tori et al. (*1*) for **1**α and **1**$\beta$, and Alaoui et al. (*15*) for **3**α. [b] Dihedral angle ∼88° for $H_4-C_4-C_5-H_5$ based on Chem 3D Pro simulation program (CambridgeSoft Corporation, Cambridge, MA). [c] Exchangeable protons with $D_2O$.

**Table 2. $^{13}C$ NMR (75 MHz) Data ($\delta$) in $CDCl_3$**

| Carbon | **1**α[a] | **1**$\beta$ | **2**α | **2**$\beta$ | **3**α[b] | **3**$\beta$ | **4**α | **4**$\beta$ | **5**α | **6** |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 29.6 | 32.6 | 33.2 | 31.0 | 29.7 | | 31.9 | 31.0 | 29.2 | 30.3 |
| 2 | 39.7 | 41.7 | 74.8 | 75.8 | 38.0 | 39.5 | 41.0 | 45.4 | 42.7 | 41.5 |
| 3 | 221.1 | 218.2 | 218.9 | 218.0 | 217.4 | 215.3 | 220.3 | 217.5 | 219.9 | 205.8 |
| 4 | 47.3 | 45.3 | 44.5 | 42.7 | 79.6 | 77.4 | 47.2 | 42.6 | 47.3 | 113.3 |
| 5 | 25.5 | 24.6 | 23.2 | 24.2 | 28.0 | 29.7 | 23.5 | 13.0 | 19.7 | 32.6 |
| 6 | 18.7 | 12.4 | 14.3 | 9.4 | 15.4 | 15.8 | 16.2 | 12.5 | 19.4 | 21.8 |
| 7 | 32.8 | 27.4 | 33.2 | 23.9 | 32.5 | 32.1 | 69.8 | 70.3 | 145.5 | 26.4 |
| 8 | 19.9 | 19.7 | 19.7 | 11.7 | 19.5 | 19.7 | 27.5 | 27.2 | 110.1 | 19.5 |
| 9 | 19.6 | 19.6 | 20.0 | 17.2 | 19.6 | 19.8 | 27.2 | 27.6 | 26.9 | 19.6 |
| 10 | 18.1 | 14.6 | 17.9 | 20.8 | 25.9 | 28.3 | 23.4 | 22.8 | 18.1 | 148.0 |

[a] Assignment based on Bohlman et al. (*16*). [b] Assignment by Alaoui et al. (*15*). Reported C-5 and C-7 chemical shifts interchanged. Present assignments are based on HMQC (heteronuclear multiple quantum coherence).

hexane to obtain pure $\beta$-thujone (>99%). Following are the sources for the other chemicals: *N*-methylmorpholine *N*-oxide, *m*CPBA, and *p*-toluenesulfonic acid, Aldrich (Milwaukee, WI); *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) and MOX reagent (2% solution of methoxyamine HCl in pyridine), Pierce (Rockford, IL). *p*-Toluenesulfonic acid was dried over $P_2O_5$ at 10 $\mu$m Hg. Oxodiperoxymolybdenum(pyridine)(hexamethylphosphoric triamide) (MoOPH) was prepared by the procedure of Vedejs and Larsen (*13*).

**Analyses.** $^1H$ NMR (300 MHz) and $^{13}C$ NMR (75 MHz) spectra were measured as $CDCl_3$ solutions on a Bruker AM-300 spectrometer using the residual $CHCl_3$ as the standard. Infrared (IR) spectra were recorded as neat samples on a Perkin-Elmer 1600 series Fourier transform IR spectrometer with bands reported as $cm^{-1}$. GC/MS was performed on a HP5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA) coupled to a HP5971 Mass Selective Detector (Hewlett-Packard) using positive chemical ionization. The GC was equipped with a DB-5 fused silica gel capillary column (30 m, 0.25 mm i.d., 0.25 $\mu$m film thickness) (J&W Scientific, Folsom, CA). The initial column temperature of 80 °C was programmed at 5 °C/min up to 200 °C, followed by an increase at 20 °C/min to 300 °C where it was maintained for 2 min. Helium was used as the carrier gas at a flow rate of 0.8 mL/min. The reagent gas was methane. Temperatures of the injection port and detector were 250 and 280 °C, respectively. All column chromatography was on silica gel.

Trimethylsilyl (TMS) derivatives were formed on reaction of alcohols with MSTFA (30 min at 80 °C) and methyloxime (MOX) derivatives were formed on coupling ketones with MOX reagent (60 min at 80 °C) (*12*). The structure of one compound (**2**α) was determined as its 2,4-dinitrophenylhydrazone derivative by X-ray crystallography. A yellow plate of dimensions 0.01 × 0.04 × 0.06 mm was the largest specimen that could be found. Data were collected using a Siemens P4 diffractometer equipped with a Siemens LT-2 low-temperature apparatus, a cold stream of 130 K, and an $\omega$ scan of 2.2° with a 1.6° offset from the center for left and right background measurements to a maximum $2\theta$ of 112° (nickel filtered Cu Kα radiation from a Siemens rotating anode source). The structure was solved by direct methods in the space group $P2_1$ and refined by full-matrix least-squares based on $F^2$ (*14*). An R1 value of 0.120 was obtained based on 408 parameters and 2516 observed data.

Hydroxythujones and dehydrothujones were synthesized as described below and characterized by $^1H$ NMR (Table 1),$^{13}C$ NMR (Table 2) and MS (Table 3).

**Preparation of 2-Hydroxythujones (2α and 2*β*) (Scheme 1).** *Preparation of* ***2***α. A 25-mL round-bottomed flask was charged with THF (2 mL) and cooled to −78 °C. A solution of *n*-butyllithium (1.6 M, 2.0 mL, 3.2 mmol) in hexane was added followed by diisopropylamine (460 $\mu$L, 3.28 mmol). The reaction mixture was stirred for 1 min and α-thujone (510 mg, 3.35 mmol) was added in THF (8 mL) over 0.5 h. The mixture was stirred for 15 min at −78 °C, warmed to −25 °C, MoOPH (2.13 g, 5.09 mmol) was added as a solid over 1−2 min with continued stirring at the same temperature for 0.5 h, and finally quenched on addition of saturated aqueous $NaHSO_3$ (10 mL). After the mixture was warmed to room temperature, saturated NaCl (10 mL) was added and the reaction mixture was extracted with ether (3 × 20 mL). The combined extracts were washed with 1N HCl (50 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated to obtain a residue which was chromatographed using 2−4% ethyl acetate in hexane to obtain **2**α (325 mg, 1.93 mmol, 58%). IR $\nu$ 3463, 2961, and 1750. The 2,4-dinitrophenylhydrazone was prepared and recrystallized (hexane/ethyl acetate) to afford yellow crystals (mp 109.5−110.5 °C).

*Preparation of* ***2***$\beta$. $\beta$-Thujone was converted to ***2***$\beta$ by the same procedure as above for α-thujone to **2**α in 61% yield. IR $\nu$ 3446, 2963, and 1748.

**Preparation of 4-Hydroxythujones (3α and 3*β*) (Scheme 2).** *Preparation of the 3,4-Enol Acetate.* α-Thujone (185 mg, 1.22 mmol) in isopropenyl acetate (1.5 mL) was treated with *p*-toluenesulfonic acid (18 mg, 0.105 mmol) and refluxed for 3 days. The reaction mixture was concentrated and chromatographed using 1−4% ethyl acetate in hexane to obtain a nonresolved mixture (220 mg, 93%) of 90% 3,4-enol acetate and 10% 2,3-enol acetate by GC/MS analysis.

*Preparation of* ***3***α. A solution of the above enol acetates (112 mg, 0.577 mmol) in $CHCl_3$ (0.5 mL) was added to a solution of *m*CPBA (75 mg) in $CHCl_3$ (2.0 mL) at 0 °C. The mixture was stirred at 0 °C for 0.5 h, then another portion of *m*CPBA (75 mg) was added, and the mixture was warmed to room temperature with continued stirring for 3 h. The reaction mixture was diluted with ethyl acetate (15 mL), then washed in sequence with 5% $NaHSO_3$ (20 mL), 5% $NaHCO_3$ (20 mL), and saturated NaCl (20 mL). The organic layer was dried over anhydrous $MgSO_4$, concentrated, and chromatographed using 1−6% ethyl acetate in hexane to obtain 4-acetoxy-α-thujone (52 mg, 43%) with the remainder mostly α-thujone. 4-Acetoxy-α-thujone (43 mg, 0.204 mmol) was dissolved in absolute methanol (10 mL), and sodium methoxide (0.1 mmol in methanol) was added at room temperature. Removal of the acetate substituent was complete after 1.5 h. A few drops of water were added, then most of solvent was evaporated under vacuum. The residue was dissolved in ethyl acetate (15 mL) and washed with water (20 mL) and saturated NaCl (20 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated to an oily residue which was chromatographed using 20% ethyl acetate in hexane to obtain **3**α as an oil (31 mg, 0.184 mmol, 90%). IR $\nu$ 3468, 2920, and 1748.

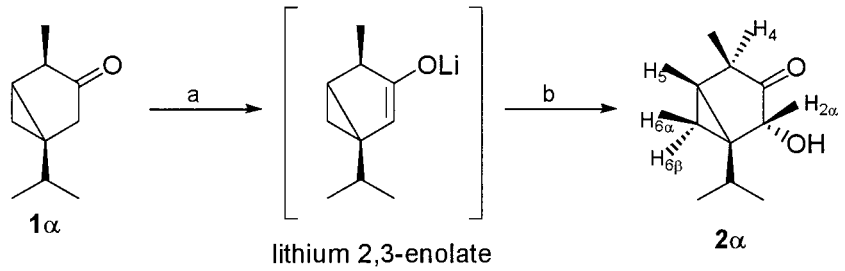
*Preparation of* ***3***$\beta$. A solution of the enol acetate isomers (100 mg, 0.514 mmol) in acetone (0.5 mL) was added to a mixture containing $OsO_4$ (10 mmol, added as a 2.5% solution in *tert*-butyl alcohol) and *N*-methylmorpholine *N*-oxide monohydrate (73 mg, 0.540 mmol) in water (1 mL) and acetone (2.2 mL) at 0 °C. The reaction mixture was stirred for 3 h at 0 °C and an additional 6 h at room temperature. $NaHSO_3$ (10 mg) was added, the pH was adjusted to 7 with 1N $H_2SO_4$, and the solvents were evaporated under vacuum. The residue was dissolved in ethyl acetate (20 mL) and washed with water (20 mL) and saturated NaCl (20 mL); then the organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was chromatographed using 10−15% ethyl acetate in hexane to obtain **3**$\beta$ as an oil (54 mg, 0.32 mmol, 62%). IR $\nu$ 3472, 2924, and 1751.

**Table 3. GC $t_R$ Values and Postulated MS Fragmentation Patterns for Thujones, Hydroxythujones, and Dehydrothujones and Their Trimethylsilyl and Methyloxime Derivatives**

| compound | $t_R$ (min) | *m/z* values of the major protonated molecular ions or fragments and their relative abundance | | | | |
|---|---|---|---|---|---|---|
| | | Nonderivatized[a] | | | | |
| | | $[MH]^+$ | $[MH-H_2O]^+$ | $[MH-H_2O-CO]^+$ | $[MH-H_2O-C_3H_6]^+$ | other |
| **1**α | 6.3 | 153(25) | 135(100) | | 93(57) | |
| **1***β* | 6.4 | 153(10) | 135(100) | | 93(45) | 123(4) |
| **5**α | 6.6 | 151(42) | 133(43) | | 93(21) | 109(40);123(100) |
| **5***β* | 7.0 | 151(42) | 133(66) | | 93(26) | 109(61);123(100) |
| **6** | 7.3 | 151(100) | | | 109(12) | 123(19) |
| **3**α | 8.0 | 169(3) | 151(100) | 123(33) | 109(87); 95(43) | |
| **3***β* | 8.2 | 169(5) | 151(100) | 123(24) | 109(59) | 95(27) |
| **2**α | 8.2 (9.6)[b] | 169(4) | 151(100) | 123(21) | 109(5) | |
| **2***β* | 9.8 (8.4) | 169(27) | 151(100) | 123(58) | 109(14) | |
| **4**α | 9.9 | | 151(100) | 123(64) | 109(12) | 95(3);168(4) |
| **4***β* | 10.1 | | 151(100) | 123(62) | 109(23) | 168(3) |
| | | TMS derivatives[a] | | | | |
| | | $[MH]^+$ | $[MH-CH_4]^+$ | [MH-TMSOH]$^+$ | other | |
| **3***β* | 11.0 | 241(15) | 225(52) | 151(100) | 109(38); 123(20) | |
| **2***β* | 11.2 | 241(13) | 225(57) | 151(100) | 123(23) | |
| **2**α | 11.3 | 241(6) | 225(81) | 151(100) | 123(8) | |
| **3**α | 12.1 | 241(8) | 225(68) | 151(100) | 109(57); 123(31) | |
| **4**α | 12.9 | 241(2) | 225(12) | 151(100) | 109(6); 123(21) | |
| **4***β* | 13.1 | 241(35) | 225(100) | 151(50) | 123(22) | |
| | | MOX derivatives | | | | |
| | | $[MH]^+$ | $[MH-OCH_3]^+$ | $[MH-H_2O]^+$ | $[MH-H_2O-OCH_3]^+$ | other |
| 1α | 8.3 | 182(100) | 150(33) | | | 96(83) |
| 1*β* | 8.7 | 182(65) | 150(32) | | | 96(100) |
| 5α | 8.8 (8.4)[b] | 180(100) | 148(53) | | | |
| 5*β* | 9.4 (9.6) | 180(100) | 148(74) | | | 107(100) |
| 6 | 9.8 (9.7) | 180(100) | 148(31) | | | 107(46) |
| **3**α | 10.8 (11.0) | | 166(61) | 180(100) | 148(28) | 71(64);124(37) |
| **2**α | 10.9 (11.1) | 198(15) | 166(54) | 180(100) | 148(45) | |
| **3***β* | 11.0 (10.9) | | 166(36) | 180(100) | 148(26) | 71(36);124(36) |
| **2***β* | 12.0 (11.6) | 198(26) | 166(64) | 180(100) | 148(32) | 138(100) |
| **4**α | 12.4 | 198(35) | 166(31) | 180(100) | 148(37) | 112(17); 138(12); 150(43); 108(20) |
| **4***β* | 12.5 | 198(18) | 166(28) | 180 (100) | 148(31) | 150(13) |

[a] The corresponding data for thujol and neothujol under the identical conditions are given in the Supporting Information. [b] Minor peaks due to thermal decomposition of 2α and 2*β* and syn and anti geometrical isomers of MOX derivatives.

**Scheme 1**[a]



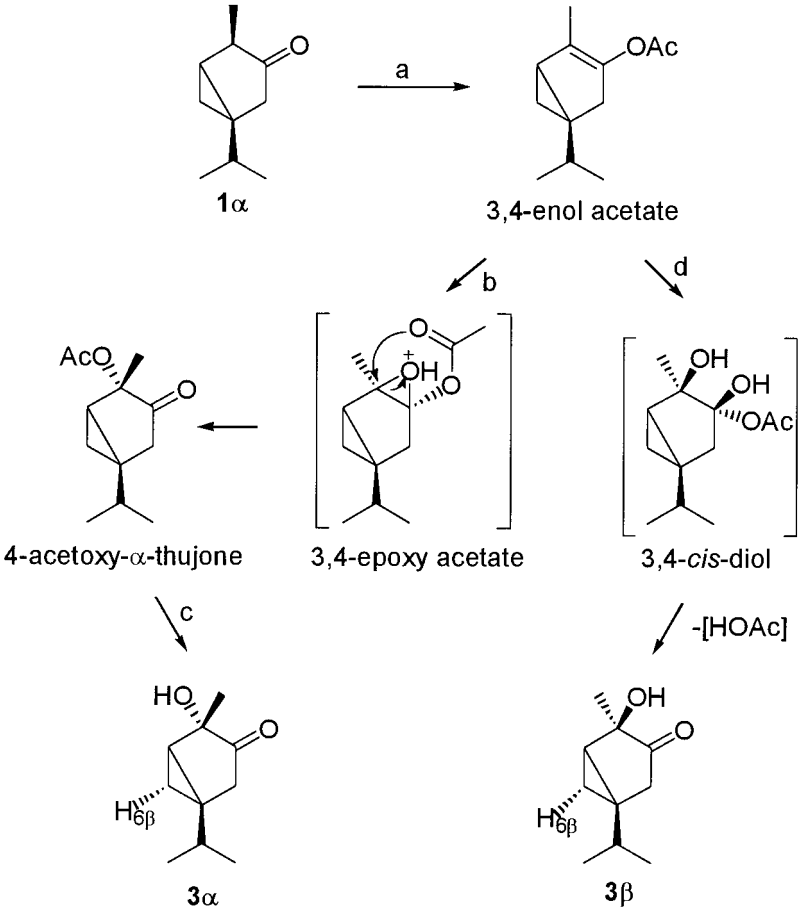

[a] (a) lithium diisopropylamide, −78 °C, THF; (b) MoOPH, THF.

**Preparation of 7-Hydroxythujones (4α and 4*β*) and 7,8-Dehydrothujones (5α and 5*β*) (Scheme 3).** *Preparation of* ***4****α and* ***4****β*. A solution of α-thujone (500 mg, 3.29 mmol) in dry ethyl acetate (50 mL) was cooled to −25 °C. A steady stream of ozone was passed though the solution for 6 h followed by a stream of dry nitrogen for 30 min. The reaction mixture was then warmed to room temperature and washed with water (50 mL) and saturated NaCl (20 mL). The organic layer was dried over $Na_2SO_4$, filtered, and concentrated. The residue was chromatographed using 20−25% ethyl acetate in hexane to obtain unreacted α-thujone (108 mg, 0.71 mmol) and **4**α (242 mg, 1.45 mmol, 44%). IR *ν* 3502, 2972, and 1733. **4**α prepared by this procedure was previously reported by Kutney and Chen (*17*). The same method was used to convert *β*-thujone to **4***β* (48%). IR *ν* 3608, 2981, and 1728.

*Preparation of* ***5****α and* ***5****β*. A mixture of **4**α (114 mg, 0.67 mmol) and pyridinium tosylate (28 mg, 0.11 mmol) in dry benzene (14 mL) was refluxed for 2.5 h using a Dean−Stark apparatus. The reaction mixture was cooled to room temperature, diluted with hexane (20 mL), and washed with water followed by saturated $NaHCO_3$ (20 mL each). The organic layer was dried over $Na_2SO_4$, filtered, and chromatographed in 3−20% ethyl acetate in hexane to obtain **5**α as an oil (34 mg, 0.226 mmol, 34%). The same procedure was used to convert **4***β* to **5***β*.

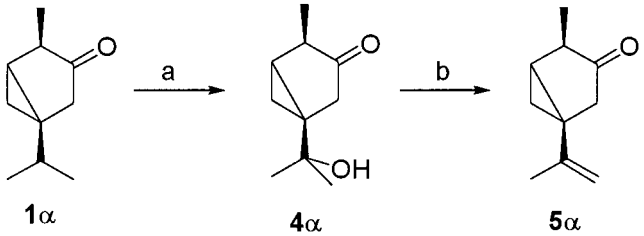
**Preparation of 4,10-dehydrothujone (6) (Scheme 4).** Sabinene (1 g) was dissolved in $CH_2Cl_2$ (3 mL), and $SeO_2$ (6 mg, 0.054 mmol) was added followed by *tert*-butyl hydroperoxide (3 mL, 90% pure). The reaction mixture was stirred overnight at room temperature, benzene (10 mL) was added and most of the solvents were evaporated under reduced pressure. Ether (20 mL) was added and the organic phase was washed with 10% KOH (3 × 20 mL) and saturated NaCl (20 mL), dried over $MgSO_4$, filtered, and concentrated. The oily residue was chromatographed using 1−6% ethyl acetate in hexane to obtain 622 mg impure sabinol. This material in $CH_2$-

**Scheme 2**[a]



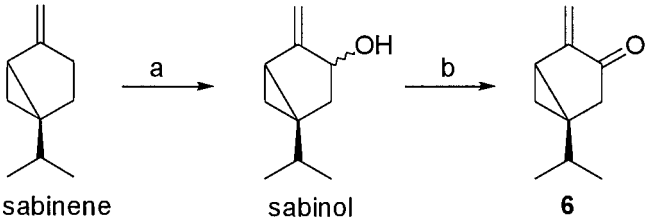

[a] (a) isopropenyl acetate, anhydrous *p*-toluenesulfonic acid, 110 °C; 2,3-enol acetate is a minor product; (b) *m*CPBA, $CHCl_3$; (c) NaOMe, MeOH; (d) $OsO_4$, *N*-methylmorpholine *N*-oxide.

**Scheme 3**[a]




[a] (a) $O_3$, −25 °C, EtOAc; (b) pyridinium tosylate or *p*-toluenesulfonic acid.

**Scheme 4**[a]




[a] (a) *tert*-butyl hydroperoxide, $SeO_2$, $CH_2Cl_2$; (b) $MnO_2$, $CH_2Cl_2$.

$Cl_2$ (16 mL) was added to a suspension of $MnO_2$ (4.5 g, 51.8 mmol) in $CH_2Cl_2$ (20 mL). The reaction was complete after 0.5 h based on TLC on silica gel. The reaction mixture was diluted with $CH_2Cl_2$ (50 mL) and filtered though a pad of Celite. The filtrate was concentrated and chromatographed using 1−4% ethyl acetate in hexane to afford 610 mg of product as a mixture. Further purification on silica gel using a Chromatotron with 1% ethyl acetate in hexane gave 320 mg of **6**. IR $\nu$ 2959, 1732, and 1637.

## RESULTS AND DISCUSSION

**Synthesis and Characterization.** The metabolites of α-thujone and β-thujone have GC/MS features, directly and on derivatization, suggesting that in each case they consist of a series of hydroxythujones and dehydrothujones (*12*). Candidate metabolites were therefore synthesized for use as standards (Schemes 1−4).

**2-Hydroxythujones (2α and 2β): Synthesis (Scheme 1) and Stereochemical Assignment.** The lithium 2,3-enolates were prepared in situ from α-thujone and β-thujone on treatment with lithium diisopropylamide at −78 °C. α-Hydroxylation of the enolate with MoOPH[13] gave a single isomeric product in both cases in good yield (58−61%). $^1$H NMR indicated retention of configuration because H-4 in **2**α is coupled only to the C-10 methyl group but not to H-5, i.e., H-4 and H-5 are orthogonal to each other. This is in contrast to **2**β where coupling between H-4 and H-5 gives rise to a multiplet for H-4 ($\delta$ 2.96). $^1$H and $^{13}$C NMR established loss of one C-2 proton and long-range coupling ($^4J_{2\alpha-6\alpha} = 2$ Hz) between H-2α and H-6α characteristic of planar zigzag orientation consistent with the **2**α and **2**β assignments. The stereochemistry of **2**α at C-2 was firmly assigned by X-ray crystallography of the 2,4-dinitrophenylhydrazone derivative (Figure 2). The unit cell contained four molecules of compound and one of water. Thus, 2-hydroxythujones from α-thujone and β-thujone are as-

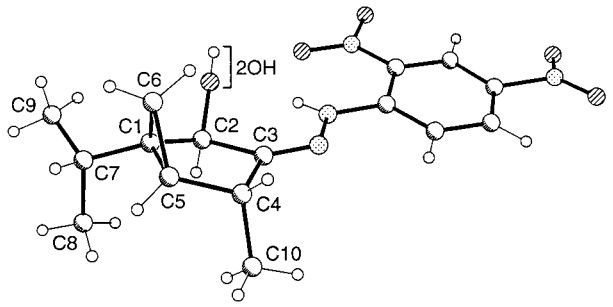



**Figure 2.** Computer-generated perspective drawing of **2**α as the 2,4-dinitrophenylhydrazone derivative based on X-ray crystal analysis. Crystallographic data are available as deposition number 153724 at the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge from CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax (+44) 1223-336-033; e-mail deposit@ccdc.cam.ac.uk.

signed as (2*R*)-2-hydroxy-α-thujone (**2**α) and (2*R*)-2-hydroxy-*β*-thujone (**2***β*), respectively.

**4-Hydroxythujones (3α and 3*β*): Synthesis (Scheme 2) and Stereochemical Assignment.** **3**α and **3***β* were prepared from the 3,4-enol acetate, obtained in high yield (93%) as a 9:1 mixture with the 2,3-enol acetate on refluxing α-thujone in isopropenyl acetate with *p*-toluenesulfonic acid using the general procedure of House and Thompson (*18*). Two different oxidants were used to prepare the final target compounds. In the first procedure the enol acetate was treated with *m*CPBA to form the 3,4-epoxy acetate in situ which quickly undergoes intramolecular rearrangement with C4−O bond dissociation to generate 4-acetoxy-α-thujone with inversion of configuration (analogous cases have been reported, *19*−*21*). The alternative pathway involving C3−O bond dissociation to generate 4-acetoxy-*β*-thujone and ultimately **3***β* was not detected. 4-Acetoxy-α-thujone was treated with 0.5 equiv of sodium methoxide to obtain **3**α in 90% yield. In the second approach the 3,4-enol acetate was oxidized with $OsO_4$ using a general method described by McCormick et al. (*22*) to obtain **3***β* in 62% yield. Product stereochemistry was assigned by two-dimensional nuclear Overhauser effect spectroscopy (NOESY) which indicated that the C-10 methyl group and H-6*β* are closer in space in the product from $OsO_4$ oxidation (**3***β*) than in the one from *m*CPBA oxidation (**3**α) (see Supporting Information).

**7-Hydroxythujones (4α and 4*β*) and 7,8-Dehydrothujones (5α and 5*β*): Synthesis (Scheme 3) and Characterization.** Ozonation of α-thujone (*17*) and *β*-thujone provided **4**α and **4***β*, respectively in 44−48% yield. Treatment of these tertiary alcohols with pyridinium tosylate or *p*-toluenesulfonic acid afforded the corresponding dehydro derivatives (34% yield for **5**α). $^1$H and $^{13}$C NMR established that the introduced hydroxy substituent is at C-7 and the double bond is at the 7,8-position.

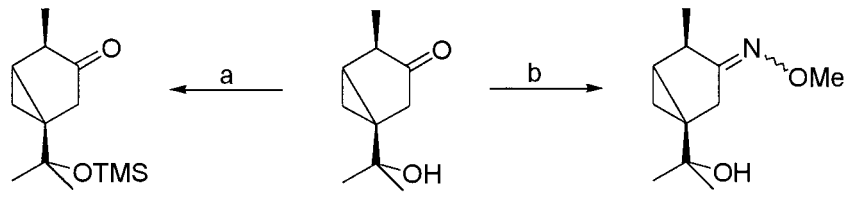
**4,10-Dehydrothujone (Sabinone, 6): Synthesis (Scheme 4) and Characterization.** Sabinene was oxidized to the allylic alcohol (sabinol) with *tert*-butyl hydroperoxide and $SeO_2$ (utilizing the general method of Umbreit and Sharpless, *23*) and then to **6** with $MnO_2$. Klinck et al. (*24*) used $SeO_2$ to convert sabinol to sabinone. The double bond is at the 4,10-position based on $^1$H and $^{13}$C NMR, and UV absorbance on TLC.

**GC/MS Analysis of Thujones, Hydroxythujones, and Dehydrothujones.** Standard analytical methods and derivatization of alcohol and ketone functionalities (Scheme 5) to TMS and MOX derivatives, respectively, were applied to α-thujone and *β*-thujone and their hydroxy and dehydro derivatives. Retention time ($t_R$) values and MS data are given in Table 3 and the fragmentation patterns are illustrated in the Supporting Information using **4**α as an example. With the nonderivatized hydroxy compounds the protonated molecular ion (*m*/*z* 169) is very small or absent because of the loss of $H_2O$, and characteristic ions are $[MH-H_2O]^+$ *m*/*z* 151, $[MH-H_2O-CO]^+$ *m*/*z* 123, and $[MH-H_2O-C_3H_6]^+$ *m*/*z* 109. The fragmentation patterns of **3**α and **3***β* show higher relative abundance of *m*/*z* 109 than do **2**α, **2***β*, **4**α, or **4***β*, suggesting a facile loss of $H_2O$ and $C_3H_6$. This difference is useful in recognizing individual metabolites in the poorly resolved **3**α plus **2**α peak (*12*). The $[MH-CO]^+$ *m*/*z* 123 fragment is characteristic for the dehydrothujones.

**GC/MS Analysis of TMS and MOX Derivatives of Thujones, Hydroxythujones, and Dehydrothujones.** With the hydroxythujone TMS derivatives, the protonated molecular ion *m*/*z* 241 is still small, and $[MH-CH_4]^+$ *m*/*z* 225 and $[MH-TMSOH]^+$ *m*/*z* 151 are common fragments. In the MOX derivatives the protonated molecular ion for α-thujone and *β*-thujone is *m*/*z* 182 versus *m*/*z* 180 for the dehydrothujones and $[MH-OCH_3]^+$ is *m*/*z* 150 and 148, respectively. With the hydroxythujone MOX derivatives the protonated molecular ion *m*/*z* 198 is more stabilized and $[MH-H_2O]^+$ *m*/*z* 180, $[MH-OCH_3-H]^+$ *m*/*z* 166, $[MH-OCH_3-OH]^+$ *m*/*z* 150, and $[MH-OCH_3-OH-C_3H_6]^+$ *m*/*z* 108 are the most common fragments.

In conclusion, all of the major metabolites (Figure 1) (*3*, *12*) have been synthesized as reported here (Schemes 1−5). Procedures to analyze them as metabolites are provided which differentiate each of the isomeric thujones, dehydrothujones, and hydroxythujones. Many of them have been tested for toxicity to insects and as blockers of the GABA-gated chloride channel with activities less than those of α-thujone and *β*-thujone, i.e., the metabolites are detoxification products (*12*). The methods of synthesis, derivatization, and analysis developed here are applicable to other monoter-

**Scheme 5**[a]




**4**α-TMS **4**α **4**α-MOX

[a] (a) MSTFA; (b) $MeONH_2$, pyridine.

penes including those used as food flavorings and seasonings.

## ABBREVIATIONS USED

*m*CPBA, *m*-choroperbenzoic acid; MoOPH, oxodiperoxymolybdenum(pyridine)(hexamethylphosphoric triamide); MOX, methyloxime reagent or derivative; MSTFA, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide; NOESY, nuclear Overhauser effect spectroscopy; TMS, trimethylsilyl substituent or derivative.

## ACKNOWLEDGMENT

We thank our laboratory colleagues Shinzo Kagabu, David Lee, Gary Quistad, Yoffi Segall, and Nanjing Zhang for helpful suggestions. Crystallography was done by Marilyn Olmstead and Håkon Hope (Department of Chemistry, University of California, Davis), and $^1$H NMR NOESY experiments were conducted by Corey Liu and Ted Barnum (Department of Chemistry, University of California, Berkeley).

**Supporting Information Available:** Figure S1 giving two-dimensional NOESY of **3**$\beta$. Figure S2 giving mass spectra of **4**$\alpha$ and its TMS and MOX derivatives using positive chemical ionization and postulated fragmentation pathways. Table S1 giving GC $t_r$ values and proposed MS fragmentation patterns for thujol and neothujol and their TMS derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

## LITERATURE CITED

(1) Tori, K. Conformations of α- and *β*-thujones. *Chem. Pharm. Bull.* **1964**, *12*, 1439−1446.
(2) Gruenwald, J.; Brendler, T.; Jaenicke, C., Eds. *PDR for Herbal Medicines*; Medical Economics: Montvale, NJ, 1998; pp 1181−1183.
(3) Höld, K. M.; Sirisoma, N. S.; Ikeda, T.; Narahashi, T.; Casida, J. E. α-Thujone (the active component of absinthe): *γ*-aminobutyric acid type A receptor modulation and metabolic detoxification. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3826−3831.
(4) Anonymous. Medicinal herbs: NTP extracts the facts. *Environ. Health Perspect.* **1999**, *107*, A604−A605.
(5) Blumenthal, M., Ed. *The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines*; American Botanical Council: Austin, TX, 1998; pp 664−665.
(6) Arnold, W. N. Absinthe. *Sci. Am.* **1989**, *6*, 112−117.
(7) Arnold, W. N. *Vincent van Gogh: Chemicals, Crises, and Creativity*; Birkhaüser: Boston, 1992; pp 101−137.
(8) Conrad, B., III. *Absinthe: History in a Bottle*; Chronicle Books: San Francisco, CA, 1988.
(9) Strang, J.; Arnold, W. N.; Peters, T. Absinthe: what's your poison? *Br. Med. J.* **1999**, *319*, 1590−1592.
(10) Vogt, D. D.; Montagne, M. Absinthe: behind the emerald mask. *Int. J. Addict.* **1982**, *17*, 1015−1029.
(11) Reese, K. M. Absinthe said to be coming back in Europe. *Chem. Eng. News* **1999**, *77*, 56.
(12) Höld, K. M.; Sirisoma, N. S.; Casida, J. E. Detoxification of α- and *β*-thujones (the active ingredients of absinthe): site specificity and species differences in cytochrome P450 oxidation *in vitro* and *in vivo*. *Chem. Res. Toxicol.* **2001,** in press.
(13) Vedejs, E.; Larsen, S. Hydroxylation of enolates with oxodiperoxymolybdenum(pyridine)(hexamethylphosphoric triamide), $MoO_5$•Py•HMPA (MoOPH): 1,7,7-Trimethyl −3-hydroxybicyclo[2.2.1]heptan-2-one (Bicyclo-[2.2.1]heptan-2-one, 3-hydroxy-1,7,7-trimethyl-), *Org. Synth.* **1986**, *64*, 127−134.
(14) Sheldrick, G. M. *Program for the Solution and Refinement of Crystal Structures;* University of Göttingen: Göttingen, Germany, 1997.
(15) Alaoui, M. I.; Benjilali, B.; Azerad, R. Biotransformation of terpenic compounds by fungi. II − Metabolism of α-(-)-thujone. *Nat. Prod. Lett.* **1994**, *4*, 263−266.
(16) Bohlmann, F.; Zeisberg, R.; Klein, E. $^{13}$C NMR-Spektren von Monoterpenen. *Org. Magn. Reson.* **1975**, *7*, 426−432.
(17) Kutney, J. P.; Chen, Y.-H. The chemistry of thujone. XVII. The synthesis of ambergris fragrances and related analogues. *Can. J. Chem.* **1994**, *72*, 1570−1581.
(18) House, H. O.; Thompson, H. W. Synthesis of $\Delta^{8,9}$- and $\Delta^{9,10}$-octal-1-one. *J. Org. Chem.* **1961**, *26*, 3729−3734.
(19) Williamson, K. L.; Coburn, J. I.; Herr, M. F. The acid-catalyzed rearrangement of enol ester epoxides. *J. Org. Chem.* **1967**, *32*, 3934−3937.
(20) Smith, S. C.; Heathcock, C. H. A convenient procedure for the synthesis of bis-steroidal pyrazines: Models for the cephalostatins. *J. Org. Chem.* **1992**, *57*, 6379−6380.
(21) Zhu, Y.; Manske, K. J.; Shi, Y. Dual mechanisms of acid-catalyzed rearrangement of enol ester epoxides: Enantioselective formation of α-acyloxy ketones. *J. Am. Chem. Soc.* **1999**, *121*, 4080−4081.
(22) McCormick, J. P.; Tomasik, W.; Johnson, M. W. α-Hydroxylation of ketones: Osmium tetroxide/*N*-methylmorpholine-*N*-oxide oxidation of silyl enol ethers. *Tetrahedron Lett.* **1981**, *22*, 607−610.
(23) Umbreit, M. A.; Sharpless, K. B. Allylic oxidation of olefins by catalytic and stoichiometric selenium dixoide with *tert*-butyl hydroperoxide. *J. Am. Chem. Soc.* **1977**, *99*, 5526−5528.
(24) Klinck, R. E.; de Mayo, P.; Stothers, J. B. Selenium dioxide oxidation product of sabinol. *Chem. Ind.* **1961**, 471−472.

Received for review December 4, 2000. Revised manuscript received February 20, 2001. Accepted February 21, 2001. This work was supported by grant R01 ES08419 from the National Institute of Environmental Health Sciences (NIEHS), NIH, and its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS, NIH.

JF001445+