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α- and β-Thujone Radical Rearrangements and Isomerizations. A New Radical Clock

Xiang He and Paul R. Ortiz de Montellano*

Department of Pharmaceutical Chemistry, University of California, 600 16th Street,
San Francisco, California 94143-2280

ortiz@cgl.ucsf.edu

Received April 21, 2004

Radical clocks have been extensively used in chemical and biochemical mechanistic studies. The C4 radicals of α- and β-thujone can undergo two distinct rearrangement reactions that could, in principle, serve as simultaneous but independent radical clocks. We have therefore generated these C4 radicals by photolysis of the corresponding *N*-hydroxypyridine-2-thione ester precursors and have investigated their fates and lifetimes. Photolysis of either α- or β-thujone generates the same 6:100 mixture of α- and β-thujone when the radicals are quenched by thiophenol. Hydrogen atom transfer from thiophenol to the radical thus occurs preferentially from the less sterically hindered α-face to give β-thujone. The third product formed in the photolysis via opening of the cyclopropyl ring is 2-methyl-5-isopropylcyclopent-2-enone. The ratio of ring opened to unopened products gives very similar values of $k_{r\alpha} = 4.4 \times 10^7$ s^{-1} and $k_{r\beta} = 1.0 \times 10^8$ s^{-1} for ring opening of the radicals generated from α- and β-thujone, respectively. If the C4 cation rather than radical is generated, it is converted to carvacrol, a phenol that is not obtained in the radical reactions. Thujone therefore differentiates between radical and cation pathways and provides a measure of the radical lifetime.

Introduction

Most alkyl radicals are thought to be either planar or slightly pyramidal with very small barriers to inversion if pyramidal[1,2] although the degree of pyramidization and the barrier to inversion are enhanced by electron-withdrawing substituents and by groups such as a cyclopropyl ring.[1,3,4] A consequence of this is that the products obtained from stereoisomeric radical precursors usually pass through a common radical species and are therefore identical. These results reflect measurements in which radicals are trapped after they reach thermodynamic equilibrium. The exploitation of conformational memory in a radical reaction has recently been reported, and the rate of loss of stereochemistry has been proposed as a new radical clock.[5] In this published work, the degree of retention of chirality in the products from radical decarboxylation of the two enantiomers of 2-(phenylmethyl)-2-carboxytetrahydropyran was used to evaluate the rate of the ring inversion reaction. The rate of racemization of the radical was calculated to be 5.7×10^8 s^{-1} at 22 °C.

Our interest in the rates of radical reactions, and particularly the use of radical clocks to determine the rates of radical reactions in enzymatic transformations, has led us to investigate the rate of cyclopropyl ring opening in the C4 radicals derived from α- and β-thujone (Figure 1). The biology of α- and β-thujone, which are diastereoisomeric monoterpenes with regard to the C4 methyl group, has been extensively investigated because of the physiological effects of α-thujone, a key active constituent of absinthe.[6] The thujone diastereoisomers have the advantage that the methyl stereochemistry can be readily determined from the ratio of easily separated products rather than from measurements of chirality. Inversion of the methyl stereochemistry of thujone has been detected in vitro and in vivo in metabolic studies, but its possible utility as a mechanistic probe has not been examined.[6] Most importantly, in addition to undergoing stereochemical inversion, the radical at C4 should also undergo a cyclopropyl ring-opening reaction for which a rate can be determined. The cyclopropyl ring-opening reaction was not observed, however, in the reported metabolic studies.[6] Thus, thujone offers the possibility of functioning as a radical probe in which the radical intermediate can be simultaneously detected by two different types of reactions. The possibility that the C4 cation would give rise to distinct products, and thus that thujone could be used to differentiate between radical and cationic pathways in enzymatic reactions, has also been investigated.

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* Corresponding author. Tel.: (415) 475-2903. Fax: (415) 502-4728.

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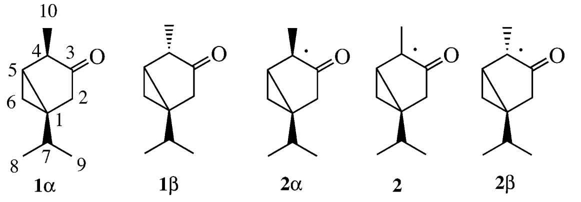

FIGURE 1. Structures of α- and *β*-thujone and their initial C4-radicals if the radical center retains nonplanar character.

N-Hydroxypyridine-2-thione esters were first reported as radical precursors in kinetic studies in 1985 and were used for measuring the rate constants for a hydrogen atom abstraction reaction in 1986.[7] Since then, many radicals have been studied for their ability to abstract a hydrogen atom from trapping agents such as thiols. For example, the fast radical rearrangements of the cyclopropylcarbinyl, bicyclo[2.1.0]pent-2-yl, and phenyl substituted cyclopropylcarbinyl radicals have been studied using *N*-hydroxypyridine-2-thione radical precursors.[8] Most studies of this kind have focused on rearrangements (e.g., the ring opening of cyclic compounds) and have been rarely used to measure the localized movements of a specific bond or group. One exception to this is the already mentioned study of conformational memory in an enantioselective radical reduction.[5] We have therefore chosen to generate the thujone radicals by converting the corresponding carboxylic acid intermediates to *N*-hydroxypyridine-2-thione esters and subjecting the products to photolytic decomposition.

Results

Synthesis of the α- and *β*-Thujone Radical Precursors. We have used the *N*-hydroxypyridine-2-thione esters of the 4-carboxylic acid derivatives of α- and *β*-thujone as the radical precursors for generating the diastereomeric C4 radicals. To prepare the *β*-thujone radical precursor, the 4-carboxylic acid derivative of *β*-thujone was synthesized from α-thujone by a literature-based two-step sequence (Figure 2). The 4-hydroxymethyl derivative of *β*-thujone (**6**) was first generated by condensing α-thujone with formaldehyde at 0 °C under basic conditions. The cyclopropyl ring sterically hinders one face of the cyclopentyl ring and forces methylation of the enolate intermediate to occur on the opposite face, resulting in inversion of the 4-methyl stereochemistry. The same product would be obtained if the reaction were carried out with *β*-thujone, as the starting stereochemistry is lost in the enolate intermediate. Subsequent Jones oxidation converted the alcohol to the carboxylic acid in 90% yield. This carboxylic acid was then converted to the *N*-hydroxypyridine-2-thione ester **8**, which was unstable to light and had to be handled in the dark. All the intermediates and products were characterized by NMR spectroscopy and by tandem GC-mass spectrometry.

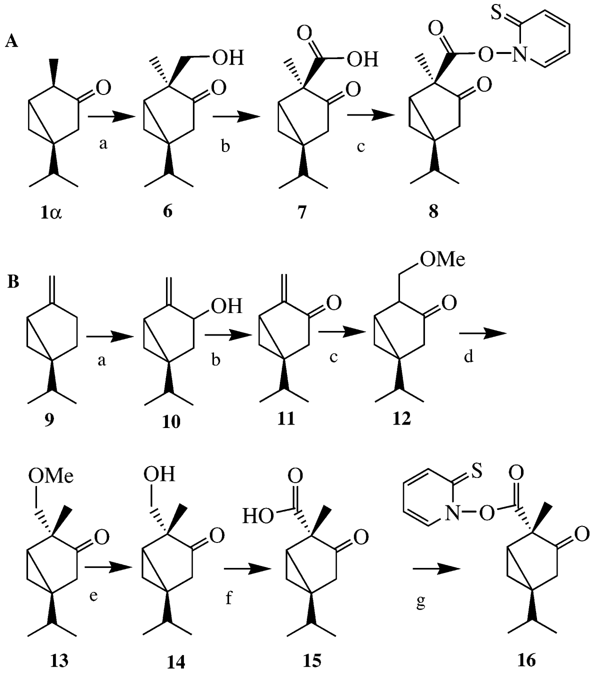

FIGURE 2. Preparation of **8** and **16**, the precursors of the α- and *β*-thujone radicals. (**A**) Synthesis of 4-[[(*β*-thujone)carbonyl]oxy]-2(1H)-pyridinethione (**8**): (a) HCHO (37%), KOH, 0 °C, 5 h; (b) Jones reagent, overnight; (c) *N*-hydroxypyridine-2-thione, DMAP, DCC, rt, 12 h. (**B**) Synthesis of 4-[[(α-thujone)carbonyl]oxy]-2(1H)-pyridinethione (**16**): (a) *tert*-butylhydroperoxide, SeO_2, CH_2Cl_2; (b) MnO_2, CH_2Cl_2; (c) NaOMe, MeOH; (d) CH_3I, NaHMDS, −78 °C; (e) BBr_3, CH_2Cl_2, −78 °C; (f) Jones reagent, overnight; (c) *N*-hydroxypyridine-2-thione, DMAP, DCC, rt, 12 h.

The diastereomeric α-thujone-4-carboxylic acid was prepared from sabinene (**9**). Sabinene was first oxidized to sabinone (or 4,10-dehydrothujone) by reaction with *tert*-butylhydroperoxide and SeO_2 followed by oxidation with MnO_2 to ensure conversion of sabinol to the conjugated ketone (Figure 2).[6b] Sabinone (**11**) was then converted to 10-methoxy-thujone (**12**) by reaction with sodium methoxide in methanol. A methyl group was subsequently introduced at C4 by submitting the enolate formed from **12** with sodium hexamethyldisilazide to reaction with iodomethane.[10] Because of the steric control exerted by the cyclopropyl group, the methylation yields almost exclusively product **13** with the methyl in the orientation found in α-thujone. The 10-methoxy group was then demethylated with BBr_3,[11] and the resulting 4-hydroxymethyl α-thujone derivative **14** was oxidized to α-thujone-4-carboxylic acid (**15)** with Jones reagent.[9,12] The carboxylic acid was finally converted to the light-unstable *N*-hydroxypyridine-2-thione ester **16**.

Radical Generation and Trapping. The *β*-thujone radical was generated and trapped by photoirradiation of precursor **8** (Figure 3) under vacuum in dry THF at 25 °C in the presence of thiophenol. Thiophenol serves as the hydrogen donor that quenches the free radical.

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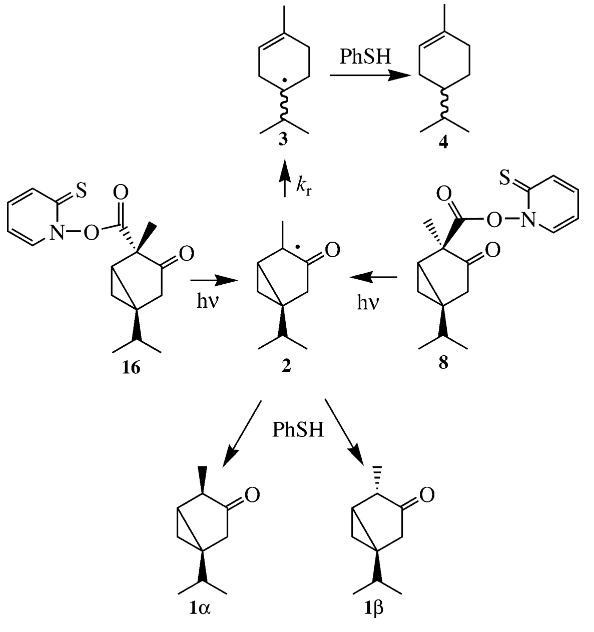

FIGURE 3. Photogeneration of the radical from **8** and **16** and the associated rearrangement reactions.

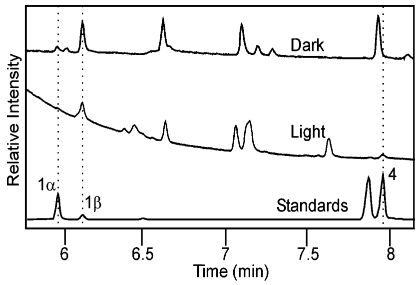

FIGURE 4. Gas chromatographic traces of photolysis experiments with 4-[[(*β*-thujone)carbonyl]oxy]-2(1H)-pyridinethione (**8**). The dark reaction reflects thermal decomposition of **8** in the inlet of the gas chromatograph. The standards are α-thujone (**1α**), *β*-thujone (**1*β***), and cyclopropyl ring opened compound **4**.

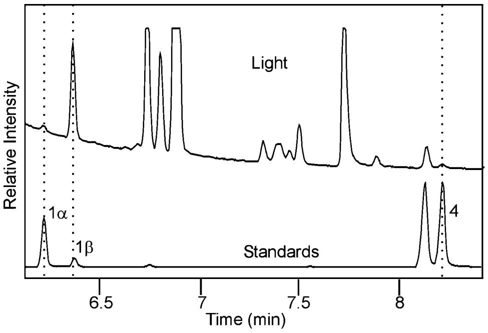

FIGURE 5. Gas chromatographic traces of photolysis experiments with 4-[[(α-thujone)carbonyl]oxy]-2(1H)-pyridinethione (**16**). The standards are α-thujone (**1α**), *β*-thujone (**1*β***), and cyclopropyl ring opened compound **4**.

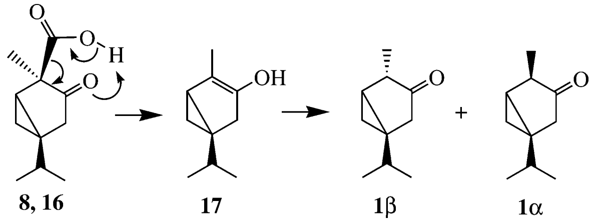

FIGURE 6. Proposed mechanism for thermodecarboxylation of **8** and **16** in the gas chromatograph inlet.

Analysis of the products by gas−liquid chromatography showed that the reaction yields α-thujone (**1α**), *β*-thujone (**1*β***), and the cyclopropyl ring opened product **4** in a 6:100:22 ratio (Figure 4). The products were identified by gas chromatographic and mass spectrometric comparison with authentic standards. A similar protocol was used to generate the α-thujone radical from the isomeric precursor **16**. In this instance, the same three products were obtained in a 6:100:11 ratio, respectively (Figure 5).

The *N*-hydroxypyridine-2-thione esters, as well as the parent α- and *β*-thujone-4-carboxylic acids, undergo thermal decarboxylation when injected into the gas chromatograph. Both the α- and *β*-thujone-4-carboxylic acids gave a reproducible α/*β* thujone product ratio of 2:98 when injected into the gas chromatograph despite clear evidence from ^{1}H NMR analysis that the starting compounds were the opposite isomers. As the decarboxylation mechanism proceeds via the enol intermediate (Figure 6), the product ratio favors the formation of *β*-thujone, as might be expected from the finding that alkylation of the enolate is favored from the α-side (Figure 2). Similarly, the *N*-hydroxypyridine-2-thione ester of α-thujone-4-carboxylic acid decarboxylates in the gas chromatograph to give an α/*β* thujone ratio of 15:100. Photolysis of the precursors therefore needs to be taken to completion to avoid errors in the product ratios caused by thermal decomposition products.

The product distribution from photolysis of **8** and **16** is determined by the rate of the cyclopropyl ring opening reaction and the rate of trapping of the various radicals by thiophenol. The observation that the same α- to *β*-thujone product ratio is obtained whether one starts with the α- or *β*-thujone radical precursor **8** or **16** is most consistent with stereocontrolled delivery of the thiophenol hydrogen from one side of the radical. In accord with the fact that an α-alkyl group is introduced by base-catalyzed alkylation regardless of whether one starts with α- or *β*-thujone, the trapping reaction apparently favors addition of the thiophenol hydrogen from the α-face. This stereoselectivity is determined by steric interference of the cyclopropyl group with hydrogen atom transfer from the exogenous donor to the *β*-face. Hydrogen addition to the α-face forces the methyl into the more sterically crowded *β*-face and yields *β*-thujone. Expressed differently, the rate k_H for H atom transfer from PhSH to the carbon radical is not the same from the α- as from the *β*-face, and the value $k_H = 2.0 \times 10^8\ M^{-1}\ s^{-1}$ at 25 °C[8b] for this reaction is not valid for addition of the hydrogen from the *β*-face. Inversion of the methyl group therefore provides evidence for a radical intermediate but cannot be used as an independent radical clock.

In contrast, the rate of the cyclopropyl ring opening reaction can be determined from the ratio of ring opened to unopened products if trapping of the unrearranged and ring opened radicals by thiophenol occurs at the rate of $2.0 \times 10^8\ M^{-1}\ s^{-1}$. This value for the trapping reaction is reasonable because it has been shown that the rates for

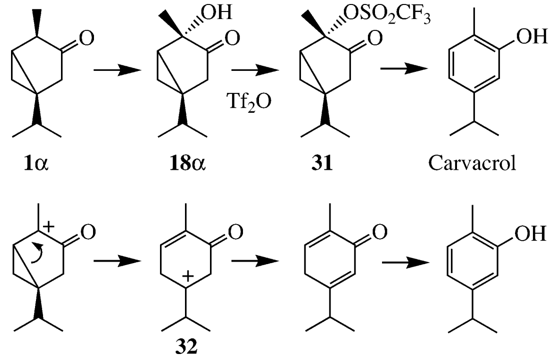

FIGURE 7. Formation and decomposition to carvacrol of the α-thujone C4 cation.

trapping of primary, secondary, and tertiary radicals are similar.[8b] The rate of the ring-opening reaction can then be expressed by the following equations, where $k_{r\alpha}$ and $k_{r\beta}$ refer to ring opening of the radicals generated from the α- and β-thujone radical precursors, **16** and **8**, respectively:

$$k_{r\alpha} = k_H[\mathrm{PhSH}][\mathbf{4}]/[\mathbf{1\beta} + \mathbf{1\alpha}]$$

$$k_{r\beta} = k_H[\mathrm{PhSH}][\mathbf{4}]/[\mathbf{1\beta} + \mathbf{1\alpha}]$$

The values obtained from these equations are $k_{r\alpha} = 4.4 \times 10^7\ \mathrm{s}^{-1}$ and $k_{r\beta} = 1.0 \times 10^8\ \mathrm{s}^{-1}$.

Thujone C4 Cation. To generate the thujone C4 cation, we attempted to make 4-tosyloxy-α-thujone for solvolytic studies. However, our synthetic efforts were unsuccessful, presumably because of steric clashes between the bulky tosyl group and the tertiary, hindered alcohol group of 4-hydroxy-α-thujone (**18α**). We therefore turned our attention to synthesis of the smaller, more reactive triflate group. The synthesis of 4-trifloxy-α-thujone was carried out in CH_2Cl_2 in the presence of 2,6-lutidine (Figure 7). In some preparations, water was added after a brief initial reaction period to allow the possible trapping of intermediates by water. The product obtained in these reactions in nearly quantitative yield was purified and shown by ^{1}H and ^{13}C NMR, UV−vis spectroscopy, and comparison with an authentic standard to be carvacrol. No more than a trace (1−2%) of the cyclopropyl ring opened product resulting from trapping of the intermediate cation **32** is observed as the single side product. This result indicates that the triflate was formed but was so reactive that it dissociated to the cation *in situ*. The cation then rearranged by ring opening of the *endo*-cyclopropyl ring, enolization, and loss of a proton to give the phenol product.

Discussion

The photolabile radical precursors **8** and **16** were obtained in workable yields by synthetic sequences that exploited the preference for alkylation of the α-face of the cyclopentyl ring opposite to that occupied by the *endo*-cyclopropyl group. The final step of these two syntheses was carried out in the dark to prevent photodecomposition of the *N*-hydroxypyridine-2-thione esters. The synthetic schemes were based on established literature methods, and the diastereomeric nature of the products was confirmed by NMR.

Photochemical decomposition of **8**, which produces the radical **2** expected from hydrogen abstraction from β-thujone, gives rise to three products: α-thujone (**1α**), β-thujone (**1β**), and the ring opened product **4** in a 6:100:22 ratio. Analogous photodecomposition of **16**, which generates the same C4 radical as hydrogen abstraction from α-thujone, gives the same three products in a 6:100:11 ratio, respectively. From these data, it is clear that the ratio of the α- and β-thujone products is determined by differential rates for trapping of the radical **2** from the two possible faces, the rate for trapping from the α-face being much faster than that from the β-face due to steric interference by the cyclopropyl ring. Because of this stereochemical control of the quenching reaction, it is not possible from the results to determine whether the C4-radical is nonplanar and exists in two conformations. In contrast, the rate for opening of the cyclopropyl ring can be determined from the ratio of ring opened to unopened products. The calculated rates for ring opening when the methyl is generated on the α-face (i.e., as in **2α**) is $k_{r\alpha} = 4.4 \times 10^7\ \mathrm{s}^{-1}$, whereas the calculated rate for ring opening when the methyl is generated on the β-face (i.e., as in **2β**) is $k_{r\beta} = 1.0 \times 10^8\ \mathrm{s}^{-1}$. These rates of cyclopropyl ring opening for the α-thujone and β-thujone radicals are very similar and are within the range of rates reported elsewhere for the opening of similar radicals.[8a,13] The similarity in the two values suggests that both are measuring reactions of the same planar radical. If the radical retains any pyramidal character, its interconversions must be rapid relative to the rate of radical trapping.

The thujone cation, in contrast to the radical, rearranges via a cyclopropyl ring opening reaction to give carvacrol (Figure 7). The cation, generated by conversion of 4-hydroxy-α-thujone (**18α**) to the triflate, was converted almost quantitatively *in situ*, in the presence or absence of water, to the aromatic product. The driving force for this transformation is release of the cyclopropyl ring strain with concomitant formation of a tertiary carbocation without an electron-withdrawing carbonyl group substituent. Enolization of the ketone and loss of a proton then leads to the aromatic phenol ring of carvacrol. The only other product observed was a trace (1−2%) of the alcohol obtained by trapping of cation **32**. We did not observe the formation of carvacrol in any photolysis experiment with either the α- or β-thujone radical.

Radical clocks have become useful tools in chemical and biochemical mechanistic studies. In the cytochrome P450 field, however, the use of radical clocks of increasing speeds has given rise to contradictory conclusions concerning the mechanism of hydroxylation of hydrocarbon C−H bonds.[14] The development of α- and β-thujone, in which two radical rearrangements occur simultaneously to give products distinct from those obtained via the cation, makes available a new tool for the study of radical intermediates in these enzymes. The thujones are currently being used to probe for radical intermediates in

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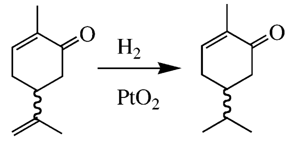

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FIGURE 8. Synthesis of carvotanacetone (**4**).

the thujone 4-hydroxylation catalyzed by cytochrome P450 enzymes.

Experimental Procedures

Materials. Unless otherwise mentioned, all reagents, organic solvents, α-thujone (**1α**), and a mixture of α- and *β*-thujone (**1*β***) were purchased from a chemical supplier. All the chemicals were used without further purification. GC and GC-MS analyses were performed on a gas chromatograph equipped with a J&W DB-1 phenyl-methylpolysiloxane capillary column. NMR spectra were obtained with a 400 MHz NMR spectrometer.

Synthesis of Carvotanacetone (4). Compound **4** was synthesized according to a published procedure[15] (Figure 8): ^{1}H NMR (400 MHz, CDCl$_3$) δ 6.75 (1H, m); 2.0−2.8 (5H, m); 1.77 (3H, s, br); 1.58 (1H, m), 0.9 (6H).

Synthesis of 8. 4-[[(*β*-Thujone)carbonyl]oxy]-2(1H)-pyridinethione (**8**) was prepared as follows according to a published procedure (Figure 2).[9]

***β*-Thujone-4-methyl Alcohol (6).**[9] *β*-Thujone-4-methyl alcohol was synthesized according to a published procedure.[9] The identity of the product (1.75 g, 95%) was confirmed by ^{1}H and ^{13}C NMR: ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.86 (1H, t, J = 5.3 Hz); 3.34 (2H, d, J = 5.3 Hz); 2.54 (1H, dd, J = 18 Hz, J = 2.3 Hz); 1.99 (1H, d, H-2α, J = 18 Hz); 1.41 (1H, m); 1.33 (1H, dd, J = 4 Hz); 0.9−1.0 (6H, 2d, J = 6 Hz); 0.87 (3H, s); 0.61 (1H, m, J = 6 Hz, J = 7.5 Hz, J = 2.3 Hz); 0.01 (1H, dd, J = 6 Hz, J = 4.5 Hz). ^{13}C NMR (400 MHz, CDCl$_3$) δ 220.5, 89.9, 68.9, 42.0, 32.5, 27.6, 27.5, 19.8, 19.6, 16.4, 15.6.

***β*-Thujone-4-carboxylic Acid (7).**[9] *β*-Thujone-4-carboxylic acid was synthesized according to a published procedure.[9] The identity of the product (1.55 g, 90%) was confirmed by ^{1}H and ^{13}C NMR: ^{1}H NMR (400 MHz, CDCl$_3$) δ 2.84 (1H, dd, J = 18 Hz, J = 2.5 Hz); 2.26 (1H, d, J = 18 Hz); 1.71 (1H, dd, J = 8 Hz, J = 4 Hz); 1.48 (1H, m); 1.28 (3H, s); 0.93−1.15 (6H, 2d, J = 6 Hz); 0.77 (1H, m, J = 6 Hz, J = 7.5 Hz, J = 2.5 Hz); 0.08 (1H, dd, J = 6 Hz, J = 4.5 Hz); −COOH not shown. ^{13}C NMR (400 MHz, CDCl$_3$) δ 211.8, 177.4, 58.8, 42.1, 32.4, 29.0, 28.2, 19.7, 19.4, 16.7, 15.7.

4-[[(*β*-Thujone)carbonyl]oxy]-2(1H)-pyridinethione (8). *β*-Thujone-4-carboxylic acid (**7**, 0.51 g, 2.6 mmol), *N*-hydroxypyridine-2-thione (0.33 g, 2.5 mmol), and *p*-(dimethylamino)pyridine (DMAP, 33 mg, 0.26 mmol) were dissolved in 25 mL CH$_2$Cl$_2$ at room temperature in a vessel shielded from light. To the mixture was added dropwise a solution of dicyclohexylcarbodiimide (DCC, 0.63 g, 2.6 mmol) in 6 mL CH$_2$Cl$_2$ over 15 min. The mixture was stirred for 12 h in the dark. Unless otherwise indicated, all the steps were conducted in the dark. The reaction mixture was filtered and concentrated. The residue was dissolved in 25 mL of ethyl acetate and sequentially washed with 10% KHSO$_4$, H$_2$O, and 5% NaHCO$_3$. The organic layer was dried over MgSO$_4$ and concentrated. The residue was purified by silica gel chromatography with 50% (v/v) ethyl acetate/hexanes as the solvent. The identity of the product (0.41 g, 55%) was confirmed by NMR: ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.66 (1H, d, J = 9.2 Hz); 7.48 (1H, d, J = 6.8 Hz); 7.19 (1H, t, J = 6.8 Hz); 3.06 (1H, dd, J = 18 Hz); 2.38 (1H, dd, J = 18 Hz); 1.70 (1H, dd, J = 8 Hz, J = 4 Hz); 1.61 (1H, m); 1.49 (3H, s); 0.95−1.09 (6H, 2d, J = 6 Hz); 0.84 (1H, m); 0.13 (1H, dd). ^{13}C NMR (400 MHz, CDCl$_3$) δ 209.4, 175.5, 166.7, 137.3, 112.6, 58.1, 42.7, 32.1, 28.4, 28.3, 19.7, 19.5, 17.2, 15.0. A high-resolution mass spectrum could not be obtained due to the light sensitivity of the compound.

Synthesis of Compound 16. 4-[[(α-Thujone)carbonyl]oxy]-2(1H)-pyridinethione (**16**) was prepared as follows (Figure 2).

4,10-Dehydrothujone (11). 4,10-Dehydrothujone was synthesized according to a published procedure.[6b] The structure of the product was confirmed by NMR: ^{1}H NMR (400 MHz, CDCl$_3$) δ 5.76 and 5.26 (2H, s, s); 2.51 (1H, dd, J = 19 Hz); 2.31 (1H, dd, J = 19 Hz); 2.02 (1H, dd, J = 8 Hz, J = 4 Hz); 1.49 (1H, m); 1.07 (1H, m); 0.94−1.00 (6H, 2d, J = 6 Hz); 0.44 (1H, dd,).

10-Methoxythujone (12). 4,10-Dehydrothujone (**11**, 2 g, 14 mmol) was dissolved in methanol (20 mL) under N$_2$. A solution of sodium methoxide (0.5 M, in methanol, 2 mL) was added, and the reaction mixture was stirred for 2 h before the reaction mixture was neutralized and concentrated. The residue was dissolved in ethyl acetate and extracted with water and saturated NaCl. The organic layer was dried over MgSO$_4$, concentrated, and chromatographed using 1−5% ethyl acetate in hexane: ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.51 (2H, t, J = 6.0 Hz); 3.32 (3H, s); 2.49 (1H, dd, J = 18 Hz, J = 2.3 Hz); 2.38 (1H, m, J = 6.0 Hz); 2.11 (1H, d, J = 18 Hz); 1.36 (1H, m); 1.31 (1H, dd, J = 4 Hz); 0.93−1.01 (6H, 2d, J = 6 Hz); 0.80 (1H, m, J = 6 Hz, J = 7.5 Hz, J = 2.3 Hz); 0.12 (1H, dd, J = 6 Hz, J = 4.5 Hz); ^{13}C NMR (400 MHz, CDCl$_3$) δ 219.1, 73.4, 58.9, 53.7, 41.7, 33.1, 29.9, 29.2, 22.0, 20.1, 19.4, 18.3; HRMS (EI, m/z) calcd. for C$_{11}$H$_{18}$O$_2$ (M$^+$) 182.1293, found 182.1292.

4-Methylenemethoxy-α-thujone (13). 10-Methoxythujone (**12**, 550 mg) was dissolved in dry THF (15 mL) at −78 °C under a nitrogen atmosphere. Sodium hexamethyldisilazide (2 mL, 2 M solution in THF) was added dropwise to the stirred solution. After 45 min, CH$_3$I (0.5 mL, passed through neutral alumina before being added) was added to the mixture over 1 min. The reaction mixture was warmed to 0 °C over 3 h before it was quenched with saturated NH$_4$Cl at 0 °C and concentrated. The residue was dissolved in ethyl acetate and washed with saturated NaCl and 10% Na$_2$CO$_3$. The organic layer was dried over MgSO$_4$, concentrated, and chromatographed on silica gel (ethyl acetate/hexanes:1:20). The yield was ~90%. The identity of the product was confirmed by ^{1}H and ^{13}C NMR: ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.39 and 3.27 (2H, dd, J = 9.6 Hz); 3.37 (3H, s); 2.66 (1H, dd, J = 18 Hz, J = 2.4 Hz); 2.10 (1H, d, J = 18 Hz); 1.41 (1H, m); 1.26 (1H, dd, J = 4 Hz); 1.18 (3H, s); 0.94−1.02 (6H, 2d, J = 6 Hz); 0.71 (1H, m, J = 6 Hz, J = 7.5 Hz, J = 2.3 Hz); 0.23 (1H, dd, J = 6 Hz, J = 4.5 Hz). ^{13}C NMR (400 MHz, CDCl$_3$) δ 219.1, 75.5, 59.6, 40.2, 32.6, 28.4, 27.0, 22.2, 19.9, 19.7, 16.3. HRMS (EI, m/z) calcd. for C$_{12}$H$_{20}$O$_2$ (M$^+$) 196.1463, found 196.1464.

α-Thujone-4-methyl Alcohol (14). 4-Methylenemethoxy-α-thujone (**13**, 530 mg, 2.7 mmol) was dissolved in CH$_2$Cl$_2$ at −78 °C under N$_2$ protection. BBr$_3$ (2.5 mL, 26 mmol) was added dropwise to the stirred solution. After 1 h, the reaction mixture was warmed to −20 °C, and the temperature was kept at −20 °C for 3 h. The reaction was then quenched very slowly with 5% NaHCO$_3$ at −20 °C. The mixture was extracted with CH$_2$Cl$_2$ (2 × 20 mL), and the organic layer was dried over MgSO$_4$, concentrated, and chromatographed on silica gel with ethyl acetate and hexanes (10% ethyl acetate/hexanes). The yield was 100%: ^{1}H NMR (400 MHz, CDCl$_3$) δ 3.72 and 3.51 (2H, dd, J = 9.6 Hz); 2.69 (1H, dd, J = 18 Hz, J = 2.4 Hz); 2.12 (1H, d, J = 18 Hz); 1.40 (1H, m); 1.10 (1H, dd, J = 4 Hz); 1.27 (3H, s); 0.95−1.02 (6H, 2d, J = 6 Hz); 0.70 (1H, m, J = 6 Hz, J = 7.5 Hz, J = 2.3 Hz); 0.27 (1H, dd, J = 6 Hz, J = 4.5 Hz); HRMS (EI, m/z) calcd. for C$_{11}$H$_{18}$O$_2$ (M$^+$) 182.1293, found 182.1293.

α-Thujone-4-carboxylic Acid (15). α-Thujone-4-carboxylic acid (**14**, 100 mg, 0.55 mmol) was dissolved in dry acetone (5 mL) at 15 °C. Jones reagent (1 mL) was added. The reaction mixture was stirred for 3 h at 15 °C (monitored by TLC) and

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[15] Dos Santos, R. B.; Zanotto, P. R.; Brocksom, T. J.; Brocksom, U. *Flavour Fragr. J.* **2001**, *16*, 303.

was then concentrated and diluted with 20 mL of water before being extracted with ether (20 mL × 3). The volume of the combined ether solution was reduced to 20 mL before extracting with 5% $NaHCO_3$ (20 mL × 4). The combined aqueous phase was slowly acidified with hydrochloric acid in a beaker and was then extracted with CH_2Cl_2 (100 mL × 3). The organic phase was concentrated and chromatographed. The yield was 100%: ^1H NMR (400 MHz, $CDCl_3$) δ 2.70 (1H, dd, $J = 18$ Hz, $J = 2.4$ Hz); 2.30 (1H, d, $J = 18$ Hz); 1.46 (3H, s); 1.44 (1H, m); 1.43 (1H, dd, $J = 4$ Hz); 0.96−1.03 (6H, 2d, $J = 6$ Hz); 0.86 (1H, m, $J = 6$ Hz, $J = 7.5$ Hz, $J = 2.3$ Hz); 0.63 (1H, dd, $J = 6$ Hz, $J = 4.5$ Hz). ^{13}C NMR (400 MHz, $CDCl_3$) δ 215.3, 175.5, 58.9, 39.8, 32.5, 29.0, 28.3, 22.8, 19.8, 19.6, 17.5; HRMS (EI, m/z) calcd. for $C_{11}H_{16}O_3$ (M^+) 196.1463, found 196.1466.

4-[[(α-Thujone)carbonyl]oxy]-2(1H)-pyridinethione (16). α-Thujone-4-carboxylic acid (**15**, 100 mg, 0.5 mmol), *N*-hydroxypyridine-2-thione (66 mg, 0.5 mmol), and *p*-(dimethylamino)pyridine (DMAP, 4 mg, 0.05 mmol) were dissolved in 10 mL CH_2Cl_2 at room temperature in a vessel shielded from light. To the mixture was added dropwise a solution of dicyclohexylcarbodiimide (DCC, 0.15 g, 0.6 mmol) in 2 mL of CH_2Cl_2 over 15 min. The mixture was stirred for 12 h in the dark. Unless otherwise indicated, all the steps were conducted in the dark. The reaction mixture was filtered and concentrated. The residue was dissolved in 25 mL of ethyl acetate and was sequentially washed with 10% $KHSO_4$, H_2O, and 5% $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel chromatography using 50% (v/v) ethyl acetate/hexanes as the solvent: ^1H NMR (400 MHz, $CDCl_3$) δ 7.68 (1H, d, $J = 9.2$ Hz); 7.56 (1H, d, $J = 6.8$ Hz); 7.19 (1H, t); 6.62 (1H, t); 2.78 (1H, dd, $J = 18$ Hz); 2.33 (1H, dd, $J = 18$ Hz); 1.67 (3H, s); 1.63 (1H, dd, $J = 8$ Hz, $J = 4$ Hz); 1.47 (1H, m); 0.95−1.09 (6H, 2d, $J = 6$ Hz; 1H, m); 0.84 (1H, dd). ^{13}C NMR (400 MHz, $CDCl_3$) δ 210.8, 175.3, 167.2, 137.6, 137.1, 133.4, 112.6, 59.1, 39.5, 32.2, 29.0, 28.7, 22.1, 19.7, 19.4, 18.3. Because of the light sensitivity of **16**, high-resolution mass spectrometric data were not obtained.

4-Trifloxy-α-thujone (31). 4-Hydroxy-α-thujone (**18α**) was prepared according to published procedures.[6b] Trifluoromethanesulfonic anhydride (100 μL, 168 mg, 0.6 mmol) was added dropwise to a stirred mixture of 4-hydroxy-α-thujone (**18α**, 50 mg, 0.3 mmol) and 2,6-lutidine (35 μL, 31 mg, 0.29 mmol) in CH_2Cl_2 (1.0 mL) at 0 °C. The reaction mixture was stirred for 10 min at 0 °C and was complete after a further 20 min at rt. Thin-layer chromatography indicated a clean quantitative reaction as the starting material was completely converted to a single product with a higher R_f value (E/H = 1:4). The reaction mixture was concentrated and chromatographed. The product was analyzed by ^1H NMR and UV−vis and shown to be carvacrol by direct comparison with an authentic sample. In some experiments, water was added at different time points during the reaction, and the organic layer was analyzed by GC-MS.

Kinetic Studies. A Pyrex tube (16 cm, 10 mm o.d.) was equipped with a stir bar, sealed with a septum, and flushed with N_2. The tube was then shielded with Al foil and cooled to −78 °C with dry ice. A stock solution of **8** or **16** in degassed THF was prepared, and an aliquot containing 8 nmol of **8** or **16** was added to the tube. A stock solution of PhSH in degassed THF was prepared, and 1 mL of this solution was added to the tube, bringing the final PhSH concentration to 2.5 M. The same results were obtained when the final concentration of PhSH was 1.0 rather than 2.5 M. The tube was then sealed under vacuum and allowed to equilibrate at rt for ∼ 2 min. The shield was then removed, and the reaction mixture was irradiated with a 150 W tungsten filament lamp for 4 h at room temperature. The tube was then cooled with dry ice before being opened. The reaction mixture was analyzed by GC using a DB-1 column, and the products were compared with authentic standards.

JO0493267