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The short synthesis of  $\alpha$ -thujone relies on the functionalization of the readily available dimethylfulvene. Furthermore, the three main metabolites of the natural product were also synthesized. Since  $d_6$ -acetone can be used as a starting material, the route developed allows for the facile incorporation of isotopic labels which are required for detecting and quantifying trace amounts via GC/MS analysis.

A great variety of plants produce the monoterpenes  $\alpha$ -thujone (**1a**) and  $\beta$ -thujone (**2**, Fig. 1), which therefore are present in diverse herbal products.<sup>1</sup> The most famous product containing thujone is certainly absinth, produced from wormwood. It had been a popular spirit drink in the 19th century but later was prohibited due to concerns about its toxicity.<sup>2</sup> It was connected to severe health problems, including hallucinations, depressions, convulsions, blindness and mental deterioration. More recent studies propose that most of these effects were caused by alcohol intoxication.<sup>2</sup> Nevertheless, thujone is neurotoxic and was shown to inhibit the gamma-aminobutyric acid A (GABA<sub>A</sub>) receptor, which leads to excitations and convulsions at higher concentrations in animal studies.<sup>3</sup>  $\alpha$ -Thujone (**1a**) was shown to be more active than its  $\beta$ -isomer **2**. The metabolism of thujone was investigated both *in vitro* and *in vivo*. 7-OH- $\alpha$ -thujone (**3a**) is clearly the major metabolite in *in vitro* studies.<sup>3</sup> *In vivo* studies, however, point to 2-OH- $\alpha$ -thujone (**4a**) and 4-OH- $\alpha$ -thujone (**5a**) as the main metabolites.<sup>4</sup>

The manufacture of thujone containing products is permitted again in the European Union but maximum limits have been imposed.<sup>5</sup> To ensure accurate quantitation, to assess whether

## A six-step total synthesis of $\alpha$ -thujone and $d_6$ - $\alpha$ -thujone, enabling facile access to isotopically labelled metabolites<sup>†</sup>

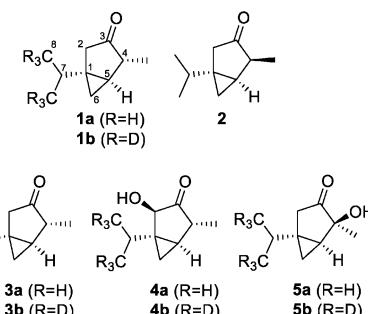
 Irene Thamm,<sup>a</sup> Johannes Richers,<sup>b</sup> Michael Rychlik<sup>a</sup> and Konrad Tiefenbacher\*<sup>cd</sup>


Fig. 1 Structures of  $\alpha$ -thujone (**1a**),  $\beta$ -thujone (**2**) and the major metabolites of  $\alpha$ -thujone: 7-OH- $\alpha$ -thujone (**3a**), 2-OH- $\alpha$ -thujone (**4a**), and 4-OH- $\alpha$ -thujone (**5a**).

these products meet the requirements and additionally to better detect trace amounts of  $\alpha$ -thujone and its major metabolites, access to isotopically labelled derivatives is required. Although the structure of the bicyclic monoterpene was elucidated in 1900 by Semmler,<sup>6</sup> only one total synthesis has been reported so far.<sup>7</sup> Oppolzer *et al.* prepared enantioenriched  $\alpha$ -thujone over twelve steps from commercially available materials, utilizing an elegant palladium-catalyzed cyclization strategy. The route, however, does not allow for a facile introduction of inexpensive isotopic labels. Therefore, we developed and herein describe a novel six-step access to  $\alpha$ -thujone, which enables the introduction of isotopic labels from inexpensive  $d_6$ -acetone. The synthesized  $d_6$ -thujone **1b** thereafter is also functionalized to the most important metabolites **3b**, **4b** and **5b**.

Our synthetic strategy is based on a late-stage oxidation of alcohol **6a/b** (Scheme 1), followed by a regio- and diastereoselective methylation at position C4. Such an approach seemed attractive since alcohol **6a/b** should be directly accessible from cyclopentenol **7a/b** via Simmons–Smith cyclopropanation.<sup>8</sup> Cyclopentenol **7a/b** can be traced back to the known dimethylfulvenes **8a** and **8b**, which are synthesized from cyclopentadiene and acetone.<sup>9</sup> Therefore, the inexpensive  $d_6$ -acetone can function as the source of the isotopic labels.

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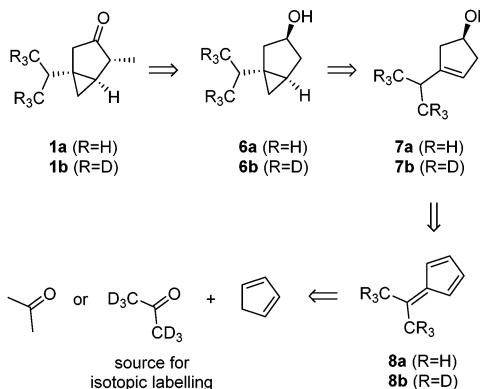
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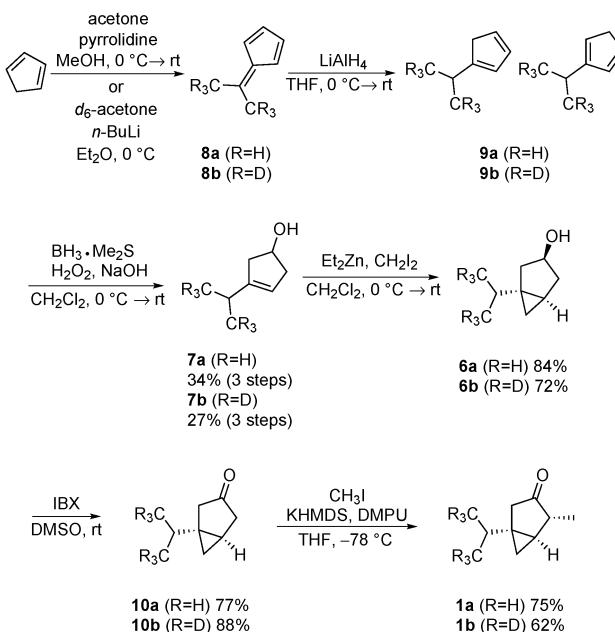
<sup>†</sup> Electronic supplementary information (ESI) available. See DOI: 10.1039/c6cc05376a





Scheme 1 Retrosynthetic analysis of  $\alpha$ -thujone (**1a**) and its deuterated derivative **1b**.

Our synthesis commenced with the formation of dimethylfulvene (Scheme 2). The preparation of **8a** followed a procedure described by Little *et al.*, utilizing pyrrolidine as a base.<sup>9a</sup> This procedure was not suitable for the preparation of the  $d_6$ -dimethylfulvene **8b**, since the deuterium label was partially removed, presumably *via* enamine formation. Therefore, the procedure introduced by the group of Gajewski was utilized to prepare  $d_6$ -dimethylfulvene **8b**.<sup>9b</sup> Using *n*-butyllithium as a base, a deuteration degree of 95.5% was achieved. The dimethylfulvenes formed were reduced with lithium aluminium hydride to yield a mixture of cyclopentadienes. It is known that related mixtures can be converted convergently to a single alcohol product *via* the hydroboration/oxidation sequence.<sup>10</sup> Indeed, such a procedure yielded, after purification by chromatography, alcohols **7a** and **7b** in 34% and 27% yield over three steps, respectively. Cyclopropanation was performed utilizing the Furukawa modification of the Simmons-Smith reaction.<sup>11</sup> After oxidation

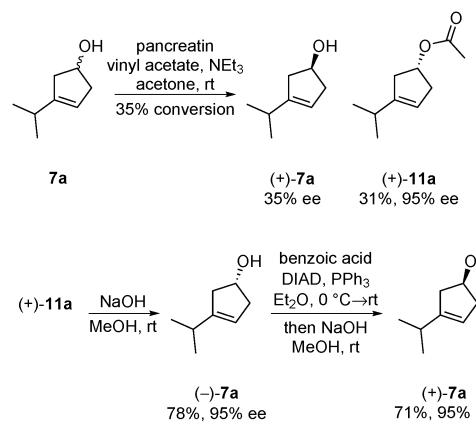


Scheme 2 Synthesis of thujone **1a/1b**.

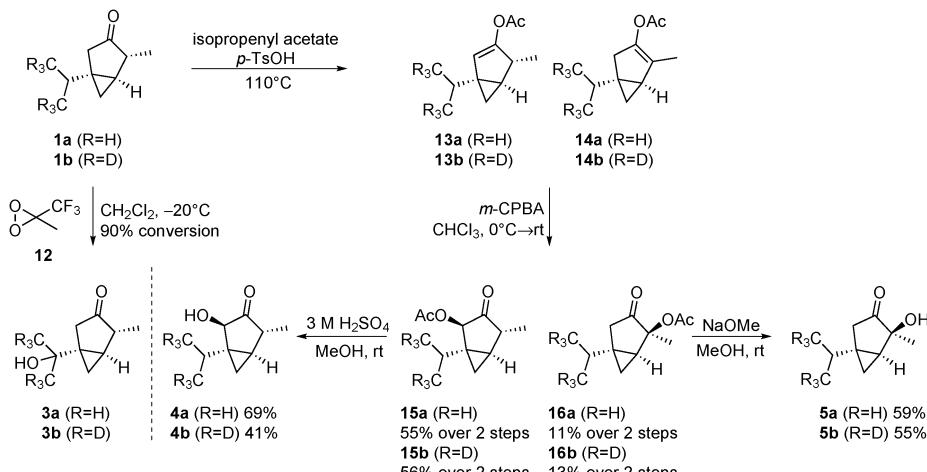
utilizing 2-iodoxybenzoic acid (IBX) as an oxidant,<sup>12</sup> ketones **10a/10b** were obtained in 77% and 88% yield, respectively. The final alkylation step required careful optimization of the reaction conditions. It was found that multi-alkylated products could not be separated by flash chromatography. Therefore, the reaction conditions were optimized to produce **1a/1b** selectively. The use of 1.0 eq. of potassium bis(trimethylsilyl)amide and methyl iodide, as well as the addition of *N,N*'-dimethylpropyleneurea, facilitated this task. Thujones **1a** and **1b** were obtained in 75% and 62% yield, respectively.

Although an optically active material is not required for use as an internal standard in GC/MS or LC/MS analysis, it may be helpful for other applications. Therefore, we investigated the possibility of performing the hydroboration of the cyclopentadienes **9a** in an enantioselective fashion. Attempts with diisopinocampheylborane<sup>13</sup> only lead to very low levels of enantiomeric enrichment (11% ee). It was therefore decided to turn to kinetic resolution. Pancreatin was utilized for the kinetic resolution of a structurally related cyclopentenol,<sup>14</sup> and also proved to be effective in this case. Kinetic resolution provided acetate (+)-**11a** in high optical purity (95% ee) and acceptable conversion (35%) (Scheme 3). Alcohol (+)-**7a**, the enantiomer required, was not accessible efficiently *via* kinetic resolution. Time-consuming repetitions of kinetic resolutions were required to increase the conversions and enantiomeric excess. It turned out to be advantageous to work with acetate (+)-**11a** and invert its stereocenter *via* the Mitsunobu reaction. This three-step procedure delivered (+)-**7a** in high optical purity (95% ee). This material was converted to optically active thujone (−)-**1a** (95% ee) as described in Scheme 2 (see the ESI†).

After having developed a concise route to thujone (**1a**) and its isotopically labelled derivative **1b**, we turned to the preparation of the most important metabolites. The oxidation of **1a** to 7-OH- $\alpha$ -thujone (**3a**) was described in the literature utilizing ozone as the oxidant.<sup>15</sup> However, these conditions lead to considerable overoxidation and a reduced yield of **3a** (47%). After screening several oxidants, we found that methyl(trifluoromethyl)dioxirane<sup>16</sup> (TFDO, **12**) led to a clean conversion to **3a** (Scheme 4). For the synthesis of the 4-hydroxy derivative **5a**, we followed the procedure of the group of Casida.<sup>17</sup> First, the



Scheme 3 Kinetic resolution of (±)-3-isopropylcyclopent-3-en-1-ol and inversion of the chiral center *via* Mitsunobu reaction.



Scheme 4 Synthesis of the hydroxylated main metabolites.

enolacetate is formed by refluxing thujone in isopropenyl acetate under acidic conditions. In contrast to the literature, we observed the 2,3-enol acetate **13a** as the main product (**13a**:**14a** = 8:2). Therefore, we decided to utilize this mixture to obtain both the 2-hydroxy and the 4-hydroxy derivative at once. The inseparable mixture was oxidized with 3-chloroperoxybenzoic acid. In both cases epoxidation was preferred from the convex bottom face, delivering after migration of the acetate group the desired diastereoisomers **15** and **16**. The isomers were separated by chromatography, and subsequently deprotected to yield the metabolites **4** and **5**, respectively. Deprotection of **15** was successful only under acidic conditions, as regular basic hydrolysis led to decomposition of the material.

In summary, we have developed a concise route to  $\alpha$ -thujone, which relies on the functionalization of dimethylfulvene. The synthesis allows for the facile incorporation of inexpensive isotopic labels by utilizing *d*<sub>6</sub>-acetone as a starting material. Furthermore, the three main metabolites of  $\alpha$ -thujone were prepared.

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