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Regioselective Benzylic Oxidation of Aromatic Abietanes: Application to the Semisynthesis of the Naturally Occurring Picealactones A, B and C


Abstract: Oxygenated aromatic abietanes are an important class of naturally occurring compounds in plants where they play specific ecological roles. However, limited availability from their natural sources hampers their exploitation for the development of new drugs to treat human diseases. Herein the benzylic oxidation of aromatic abietanes mediated by sodium chlorite and aqueous tert-butyhl hydroperoxide is reported. The method is regioselective for 12-substituted derivatives and gives the 7-oxo products, in good yields. Moreover, it conveniently replaces the use of toxic chromium reagents for this transformation. Preparation of 7-oxo, 7-oxo-15-hydroperoxy and 7-oxo-15-hydroxy derivatives of other aromatic abietanes is also possible with this method. Furthermore, the reaction products were used as key intermediates for a short and facile semisynthesis of the naturally occurring picealactones A, B and C to disclose their anti-proliferative activity for the first time.

Introduction

The oxidation of benzylic C-H of aromatic abietanes such as methyl dehydroabietate 1 (Table 1) is a key chemical transformation extensively investigated in the literature. Oxygenated aromatic abietanes at positions 7 and 15 exist widely in nature and display important biological activities including, for instance, the 7-oxo compounds sugiol and salvinolone, the 7-hydroxy aquilarabietic acids, the 7,20-lactone carnosol as well as podocarpic acids, which are oxidized at positions 7 and 15, or by podocarpane-type diterpenoids.

15-hydroxy compound angustanoic acid F, the 7-oxo-15-hydroxy and 7-oxo-15-hydroxy compound angustanoic acid F. Like many other natural products, they are usually not available in large amounts and paper, as well as water disinfection. In organic chemistry, oxidation systems based on sodium chlorite or hypochlorite and TBHP are known as inexpensive and environmentally friendly water-based oxidation methods. Inorganic Chemistry, Faculty of Pharmacy

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[b] Vanessa Brito, Dr. Samuel M. Silvestre

[c] Dr. Tobias Rüffer, Prof. Dr. Heinrich Lang

[d] Dr. Samuel M. Silvestre

[e] Dr. Vânia M. Moreira
Results and Discussion

The oxidation of 1 with NaClO₂ and aqueous TBHP was first examined according to the conditions reported on Table 1, Entry 1. A mixture of products was formed which could not be easily separated by column chromatography. However, it was possible to identify the 7-oxo 2a, 15-hydroperoxy 2c and 15-hydroxy 2d derivatives. The major reaction product 2a was present in a mixture with the 7-hydroperoxy derivative 2b, identified by the position of the C7 signal on the 13C NMR spectrum at 81.5 ppm. Extension of the reaction time to three days (Table 1, Entry 2) gave products 2a, 2c and 2d which were isolated by column chromatography in 39%, 31% and 7% yields, respectively. Using these conditions, total consumption of the 7-hydroperoxy derivative 2b allowed the isolation of pure 2a and also resulted in the formation of higher amounts of products 2c and 2d. As hydroperoxides such as 2c are usually relatively unstable, the reaction was next carried out under the same conditions other than the washing with the reducing agent Na₂SO₃ during the work-up (Table 1, Entry 3). No significant changes were observable. However, the work-up and purification became more difficult due to a poorer elimination of the remaining oxidant and therefore the time of washing with Na₂SO₃ was set to 3 hours with magnetic agitation. Of note, preparation of the 15-hydroxy derivative 2d, in high yields (~ 90%), is possible from 2c by treatment with either trimethyl phosphate or triphenyl phosphine, in dichloromethane.

We also carried out the reaction in the presence of either NaClO₂ or TBHP as single reagents (Table 1, Entries 4-5). After 3 days the reaction failed to proceed and the starting material could not be exhausted, thus revealing the need for the combination of both reagents for an effective oxidation of 1. The same occurred when NaClO₂ was tested in catalytic amounts (Table 1, Entry 6). Manipulation of the amounts of oxidant vs time of reaction (Table 1, Entries 7-8) resulted in a shift in the product ratio towards the formation of the hydroperoxide 2c and slightly higher amounts of 2d. However, with very high amounts of oxidant (4.2 equiv.) and extension of the reaction time to 7 days, a mixture of chromatographically non-separable products formed (data not shown). Previous studies report a single preparation procedure for 2c with an O₂/NHPI system combined with either cobalt(II) catalyst or V-70, and high yields were attained only when the 7-oxo derivative 2a was used as the starting material, i.e., with two sequential oxidations. Attempts to oxidize the 7-oxo derivative 2a with the NaClO₂/TBHP system not only failed to exhaust the starting material but also resulted in an inseparable mixture of various oxidation products. Also, the direct oxidation of 1 with catalytic amounts of NHPI in combination with NaClO₂ (1.2 equiv.), resulted in reduced yields of the oxidation products 2a (24%), 2c (3%) and 2d (4%) along with 13% of the starting material. Overall, the reaction conditions described in Table 1, Entry 2, allowed the easy preparation and isolation of products 2a and 2c, along with minor amounts of 2d.

Table 1. Oxidation of methyl dehydroabietate 1 with NaClO₂/TBHP in acetonitrile/water (3:1)

<table>
<thead>
<tr>
<th>Entry[a]</th>
<th>NaClO₂ (equiv.)</th>
<th>TBHP (equiv.)</th>
<th>Time (days)</th>
<th>Yield (%)</th>
<th>Ratio of products</th>
</tr>
</thead>
<tbody>
<tr>
<td>1[b]</td>
<td>1.2</td>
<td>5.0</td>
<td>1</td>
<td>31[c]</td>
<td>12[d] 10 2</td>
</tr>
<tr>
<td>2[b]</td>
<td>1.0</td>
<td>5.0</td>
<td>3</td>
<td>39</td>
<td>0 31 7</td>
</tr>
<tr>
<td>3[b]</td>
<td>1.0</td>
<td>5.0</td>
<td>3</td>
<td>32</td>
<td>0 25 8</td>
</tr>
<tr>
<td>4[b]</td>
<td>1.0</td>
<td>5.0</td>
<td>3</td>
<td>29</td>
<td>-- -- --</td>
</tr>
<tr>
<td>5[b]</td>
<td>0</td>
<td>5.0</td>
<td>3</td>
<td>traces</td>
<td>-- -- --</td>
</tr>
<tr>
<td>6[b]</td>
<td>0.1</td>
<td>5.0</td>
<td>3</td>
<td>traces</td>
<td>-- -- --</td>
</tr>
<tr>
<td>7[b]</td>
<td>1.5</td>
<td>5.0</td>
<td>7</td>
<td>23</td>
<td>0 34 9</td>
</tr>
<tr>
<td>8[b]</td>
<td>2.4</td>
<td>5.0</td>
<td>2</td>
<td>28</td>
<td>0 35 12</td>
</tr>
</tbody>
</table>

[a] All reactions were carried out with 0.500 g (1.59 mmol) of 1. [b] 20 hours of Na₂SO₃ wash. [c] Products 2a and 2b isolated as a mixture. Product yields estimated based on the NMR spectrum. [d] Washing with Na₂SO₃ was set to 3 hours with agitation. [e] No Na₂SO₃ washing during the work-up procedure. [f] No exhaustion of the starting material.
As 1 remains to date the only extensively studied starting material for the benzylic oxidation of dehydroabietanes, we tested the reaction on other dehydroabietic acid derivatives (Table 2). We found that the method was suitable for the oxidation of primary 3, secondary 5 and tertiary amides 7 and the oxidation pattern was similar to the one observed with methyl dehydroabietate 1. The oxidation of 3 formed slightly smaller amounts of 7-oxo-15-hydroperoxy product 4b than that of 5 and 7, suggesting that 4b is probably less stable.

![Table 2: Oxidation of primary 3, secondary 5 and tertiary 7 amide derivatives of dehydroabietic acid with NaClO$_2$/TBHP](image)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>R</th>
<th>Product</th>
<th>Yield (%)</th>
<th>Ratio of products</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>NH$_2$</td>
<td>4a</td>
<td>30</td>
<td>58:17:25</td>
</tr>
<tr>
<td>5</td>
<td>NHCH$_2$CO$_2$Me</td>
<td>6a</td>
<td>38</td>
<td>66:26:9</td>
</tr>
<tr>
<td>7</td>
<td>NMe$_2$</td>
<td>8a</td>
<td>35</td>
<td>52:39:9</td>
</tr>
</tbody>
</table>

[a] All reactions were carried out with 0.500 g of the starting material.

To further investigate the scope of this method, we attempted next the direct oxidation of 9 (Scheme 1). Surprisingly, only the 7-oxo derivative 10 was obtained, in 42% yield, after purification by column chromatography. We reasoned that the high polarity of the oxidation products formed, which hampers chromatographic purification, as well as the partial degradation of 9 during the reaction, account for the observed results and yield. Nonetheless, the fact that compound 9 could be directly oxidized with this method to give a single reaction product, albeit in moderate yield, is encouraging as previous studies report only the use of chromium(VI)-based reagents for this transformation.

A free radical mechanism is generally accepted for hydroperoxide- and chlorite-mediated allylic/benzylic oxidations and a determinant step is the formation of carbon radicals which can further react to form oxygenated products.[20,21] To confirm the reaction mechanism, an experiment was run with the conditions in Table 1, Entry 2, in the presence of the radical inhibitor butylated hydroxytoluene (BHT, 50 mol%). After 3 days, starting material was recovered along with small amounts (14%) of the 7-oxo product 2a, clearly indicating that the oxidation is hampered by the presence of BHT.

In the course of this study, we consistently observed a higher reactivity at C7 for compounds 1, 3, 5, and 7 that differ in the functional group present in ring A. Therefore, we addressed the effect of ring C substitution on the reaction outcome next by oxidizing the 12-substituted compounds 11, 13, 15, and 17. As shown in Scheme 1, during the oxidation of the 12-acetyl derivative 11, a single product formed that corresponded to the 7-oxo derivative 12, and was isolated in 81% yield. The observed regioselectivity and yield after chromatographic purification were remarkable as oxidation of this compound with different amounts of CrO$_3$ (3.0-3.5 equiv.) fails to provide compound 12 in yields higher than 53%. The reaction was further tested on the 12-acetyl derivatives 13 and 15 and the respective 7-oxo products 14 and 16 were isolated as the single reaction products, in 52% and 51% yields, respectively, after chromatographic purification. Finally, the 12-methoxy derivative 17 was prepared to verify whether the outcome of the reaction would change by introduction of an electron-donating group at C12. However, the oxidation of 17 also gave a single 7-oxo product 18 (Scheme 1).

To account for the observed regioselectivity, a theoretical computational study was made where the stability of the...
dehydroabietyl radicals at C7 and C15 of compounds 1, 11 and 17 was calculated by ab initio computational studies at the B3LYP/6-31G(2d,p) level (Table 3). The study revealed that the free energy at 298 K of the C7 radical is lower than that of the C15 radical which can explain the preferential oxidation at C7. Moreover, the energy difference between the C7 and the C15 radicals is much higher for compounds 11 and 17 than for compound 1, which supports the observation that only the 7-oxo derivatives are formed in the case of the 12-substituted substrates.

Table 3. Thermochemical calculations of model radicals free energies (values reported for Gibbs energy at 298 K)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative stability of C7 vs C15 radical (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-2.003</td>
</tr>
<tr>
<td>11</td>
<td>-7.917</td>
</tr>
<tr>
<td>17</td>
<td>-7.009</td>
</tr>
</tbody>
</table>

As depicted in Scheme 2, after preparation of intermediates 10 and 2d by means of the NaClO2/TBHP oxidation, the picealactones A 20 and B 22 were prepared, in a sequential synthesis, using a modification of the procedure of Korovin et al. by bubbling air into the reaction mixture containing the respective substrates in the presence of potassium tert-butoxide in tert-butanol. The resulting crude reaction mixture contained diosphenol 19 (21) and a small amount of the desired lactone 20 (22) which after treatment with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) hydrochloride, gave the desired picealactones A (20) and B (22) as single products (54% and 55% yields, respectively, after purification by column chromatography). Overall, the picealactones A 20 and B 22 were synthesized from the commercially available starting material 9 in 3 and 4 steps, respectively, and their structures were confirmed by single crystal X-ray studies after recrystallization from methanol. Picealactone C (25) was synthesized similarly by bubbling air into the reaction mixture of 18 and potassium tert-butoxide in tert-butanol giving a mixture of 23 and 24 which was treated with EDC hydrochloride to give 24 as a single reaction product. Hydrolysis of 24 with boron tribromide gave the desired picealactone C (25) in a total yield of 39%, over 4 steps (Scheme 3). A screening of the anti-proliferative properties of the picealactones using prostate (PC-3) and breast (T47D) cancer cell lines revealed that picealactones A (20) and C (25) inhibited the proliferation of breast cancer cells lines with IC50 values close to 10 μM and can be regarded as good starting materials for further optimization in pursuit of new agents to target endocrine cancers (Supporting Information).

The utility of this procedure for the semisynthesis of the naturally occurring picealactones A, B and C was next demonstrated. These picealactones were isolated from the heartwood of Picea morrisonicola Hayata and bear a 5-dehydro-18,6-olide moiety common to the ent-kaurane stevionolide from Stevia lucida Llag. A previous report concerns their preparation from a bietic acid but their biological activity has not been investigated to date.

![Scheme 2](image-url)
Scheme 3. Synthesis of picealactone C (25). Reagents and conditions: a) NaClO₂, TBHP, MeCN/H₂O (3:1), 60 °C, 3 d, 74%; b) air, t-BuOK, t-BuOH, 35 °C, 3.5 h; c) EDC hydrochloride, DMAP, CH₂Cl₂, 0 °C → rt, 2.5 h, 71% (yield over 2 steps); d) BBr₃, CH₂Cl₂, 0 °C, 6 h, 75%.

Conclusions

In summary, the NaClO₂/TBHP system is a convenient, eco-friendly and inexpensive method for the preparation of 7-oxo, 7-oxo-15-hydroperoxy and 7-oxo-15-hydroxy derivatives of aromatic abietanes. It allows the direct oxidation of several dehydroabietanes bearing different functional groups such as carboxyl, carbonyl, amide and methoxide, via a radical-mediated reaction mechanism. Moreover, for 12-substituted aromatic abietanes, the method is regioselective and gives the 7-oxo derivatives exclusively, in good yields. This work conveniently replaces chromium(VI)-based reagents for these transformations and provides an easy access to key reaction intermediates that can be used in the semisynthesis of naturally occurring diterpenoids, otherwise inaccessible for further studies in the development of new drugs. We believe our work will be considered of general utility and become widely adopted by those working in the field of diterpenoids chemistry.

Supporting Information Summary

Compound spectra and experimental procedures. Thermochemistry, single crystal X-ray crystallographic data and MTT assay data.

Acknowledgements

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Keywords: abietic • benzylic oxidation • eco-friendly • picealactone • regioselective

Benzylic oxidations: The regioselective benzylic oxidation of aromatic abietanes is accomplished by a system comprising of sodium chlorite and aqueous tert-butyl hydroperoxide. The method obviates the need for chromium(VI)-based reagents for this transformation and provides an easy access to versatile intermediates for the synthesis of bioactive diterpenoids.