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Citation for final published version:


Publishers page: http://dx.doi.org/10.1002/anie.201808605 <http://dx.doi.org/10.1002/anie.201808605>

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Short Total Synthesis of Ajoene

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Abstract: We describe a short total synthesis of ajoene, a major biologically active constituent of garlic. The instability of allicin as the only other known alternative starting material has led to the development of a reliable procedure for the synthesis of ajoene from simple building blocks that is also suitable for upscale operations.

For a long time, garlic extracts and garlic-based products have been used worldwide not only as food ingredients, but also as medicine for the prevention of stroke, coronary thrombosis, and atherosclerosis, as well as in the treatment of infections and vascular disorders.[1] The therapeutic benefits of garlic are manifold and relate to the high concentrations of organosulfur compounds present in this plant. However, the instability of the major component allicin (1) limits the commercial viability of garlic extracts. Among other constituents of garlic, ajoene (2) derived from allicin is biologically active and more stable.[2]

To the best of our knowledge, there is only one reported synthesis of ajoene (2). Block and co-workers described the biomimetic thermal rearrangement of allicin in aqueous acetone (Scheme 1),[3] and recently this synthesis has been extended to produce a trifluorinated analogue.[4] Although the synthesis is a one-pot conversion, it suffers from low yields (34%) owing to the formation of reactive sulfur-containing intermediates that also lead to side products. This reaction also does not allow for the synthesis of structurally modified or substituted analogues. More recently, Hunter and co-workers reported a synthetic route to prepare a range of ajoene derivatives but this route could not be used to synthesize ajoene 2 itself.[5]

We present here an efficient total synthesis of ajoene (2). An isothiouronium salt was prepared by reaction of bromide 3 (R = OH) with thiourea, which was then hydrolyzed to the thiol and propargylated to form the thioether 4. The reaction of the hydroxy group in 4 with 2-nitrophenyl selenocyanate and tributylphosphine produced the selenide 6a (Scheme 2).

Alternatively, dibromide 3 (R = Br) can be treated with the phenylselenenyl anion generated in situ from diphenyl diselenide to afford bromide 5. Compound 5 was then used to synthesize the propargylthioether 6b using the same sequence of isothiouronium salt formation, hydrolysis, and propargylation. The overall yields for the reaction sequences to 6a and 6b are 29% and 63%, respectively. The selenium moiety will serve as the handle to introduce an alkene through a selenoxide elimination.

The next step of the synthesis involved the regioselective addition of thiocetic acid to the terminal alkene 6. The reaction was carried out by dissolving alkene 6 in degassed toluene and heating to 85°C with a radical initiator added to the solution, followed by the dropwise addition of thiocetic acid over 40 min using a syringe pump (Scheme 3). When ACCN [azobis(cyclohexanecarbonitrile)] was used as the radical initiator, compound 7a was obtained as a 2:3 mixture.

Scheme 1. Block’s synthesis of ajoene (2).


Scheme 3. Radical addition of thioacetic acid to form derivatives 7.
of the E/Z stereoisomers in 50% yield (7b: 2:3 E/Z, 64%). For 7b, the yield was slightly improved to 71% when AIBN [azois(isobutyronitrile)] was used instead of ACCN. We could show that at this stage, the separation of the E and Z stereoisomers was possible by chromatography. However, the use of the next reaction as it is not stereospecific.

The hydrolysis of 7 to the thiolactone was achieved with potassium hydroxide in methanol, and the subsequent sulfinylation with thiosulfonic acid S-alkyl ester 8F7 occurred in good yields to give compound 9 (Scheme 4). The reaction was performed at −40°C in order to avoid side reactions of the highly reactive thiolactone. The reaction with compound 7a (2:3 mixture of E/Z stereoisomers) afforded 9a in 73% yield with the same E/Z ratio. When the reaction was performed with compound 7b (1:1 mixture of E/Z stereoisomers), compound 9b was obtained in 87% yield and the ratio of E/Z stereoisomers changed to 2:3. As the stereoisomers could be separated by chromatography, a reaction with (Z)-7b was performed to determine whether isomerization to the E isomer occurs at even very low temperatures. Indeed, the reaction afforded 9b as a 3:2 mixture of E/Z stereoisomers in 85% yield.

In the final step of the synthesis, compound 9 was treated with two equivalents of 30% w/w hydrogen peroxide solution to form ajoene 2 in a 2:3 mixture of E/Z stereoisomers in 27% (9a) and 23% (9b) yield. The selenide as well as the sulfide functional group were oxidized while the selenoxide undergoes a direct selenoxide elimination to form a double bond, the sulfoxide is retained in the product molecule. The syn elimination of alkyl aryl selenoxides is an efficient synthetic procedure to form alkoxenes. It is known that electron-withdrawing substituents on the aromatic ring increase both the rate of elimination and the yield of the alkene.58 However, the use of the selenium derivative with an electron-withdrawing substituent, 9a, did not show any advantage when compared with compound 9b as the yields were almost identical. A perselenenic acid byproduct might be able to catalyze the oxidation to the sulfoxide.59 The yields for the conversion from 9 into ajoene 2 were rather low on 0.3 mmol scale.

Further optimization studies of the selenoxide elimination and concomitant sulfur oxidation were carried out. For this study, compound 7a was used as the model substrate to find suitable reaction conditions. Product 10 can also be used as an ajoene precursor. Different oxidation conditions were investigated, and the results are presented in Table 1. The reaction of compound 7a with 2 equivalents of H2O2 (50% w/w) afforded products 10 (23%) and 11 (19%; Table 1, entry 1). Increasing the amount of oxidant to 3 or 4 equivalents did not improve the yield of compound 10, and compound 11 was still isolated (Table 1, entries 2 and 3). The complex urea hydrogen peroxide (UHP) was also used as an alternative to aqueous hydrogen peroxide solution. The reaction of compound 7a with 2 equivalents of UHP afforded, aside from compound 10 in 33% yield, compound 11 in 17% yield (Table 1, entry 4). Interestingly, when the reaction of compound 7a was carried out in the presence of NaIO4, compound 11 was isolated as the major compound in 50% yield (Table 1, entry 5). meta-Chloroperbenzoic acid (mCPBA) was also investigated as a suitable oxidant and found to give comparable yields (Table 1, entry 6). Independent of the reaction conditions, compounds 10 and 11 were the two major products formed and isolated. However, smaller amounts of other non-identified side products were also detected. Areneselenenic acid generated during the selenoxide elimination is in equilibrium with its disproportionation products (diaryl diselenide and areneselelenic acid). Under neutral or acidic conditions, they can react with alkene to generate side products. These side reactions can be suppressed by the addition of alkyl amines. When 1.5 equivalents of diisopropylamine (DIPA) were added to the reaction with H2O2, the formation of compound 11 was suppressed, but compound 10 was only isolated in 27% yield. Adding 2 equivalents of DIPA to the reaction with mCPBA not only stopped the formation of compound 11, but also improved the yield of product 10 to 46% (Table 1, entry 8). Increasing the reaction time under the same reaction

### Table 1: Optimization studies.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Oxidation conditions</th>
<th>Yield [%]</th>
<th>7a</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2 equiv H2O2 (50% w/w), THF</td>
<td>20</td>
<td>23</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3 equiv H2O2 (50% w/w), THF</td>
<td>21</td>
<td>20</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4 equiv H2O2 (50% w/w), THF</td>
<td>12</td>
<td>9</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2 equiv UHP, CH2Cl2</td>
<td>6</td>
<td>35</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2 equiv NaIO4, CH3OH/H2O</td>
<td>9</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2 equiv m-CPBA, CHCl3</td>
<td>37</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2 equiv H2O2 (50% w/w), CH2Cl2</td>
<td>20</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.5 equiv DIPA, 0°C (1 h)–rt (2 h)</td>
<td>46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2 equiv m-CPBA, CH2Cl2, 2 equiv DIPA</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Scheme 4. Synthesis of ajoene (2).](image-url)
conditions did not affect the yield of compound 10 (Table 1, entry 9).

The complete synthesis of ajoene 2 was scaled up, with slightly different results being obtained.\[10\] The synthesis of 5 proceeded with 58\% yield on 4 mol scale while the subsequent thiol formation and propargylation led to 6b in 87\% yield (2.9 mol). Radical addition of thioacetic acid proceeded similarly well compared to the small-scale synthesis (7b: 75\%, 1.4 mol) as did the thioacetate cleavage and thioallylation to 9b (74\%, 1.1 mol). The final oxidation to ajoene 2 displayed superior yields (65\%) compared to the small-scale synthesis, and 169 g (0.72 mol) of ajoene 2 were isolated in about 90\% purity as determined by HPLC and NMR analysis.

Much of the research interest in ajoene 2 resides in its biological activity. It has been shown to have efficacy in a number of biological studies, including antithrombotic and antifungal activities.\[13\] In order to further evaluate 2, its activity in a biological assay was also examined. Ajoene’s ability to act as a quorum sensing inhibitor (QSI) was selected as this is one of its more recent remarkable biological properties. Quorum sensing (QS) is a mechanism of cell–cell communication in bacteria facilitated by the secretion and detection of signaling molecules such as N-acetyl homoserine lactones in Gram-negative bacteria.\[12\] QS allows bacteria to synchronize specific gene expression, which has an impact on their pathogenicity and is thought to play a significant role in the formation of biofilms. Recent studies have shown that ajoene 2 is an effective QS inhibitor against Pseudomonas aeruginosa and Staphylococcus aureus and could be utilized for the treatment of chronic biofilm infections by exploiting the QS system.\[13\] In this study, we employed a reporter strain (Pseudomonas aeruginosa Pa01 lasB-gfp)\[13\] whereby QS gene expression was monitored over time in response to ajoene treatment.

Two ajoene products were examined: 2 (synthetic) as synthesized above and ajoene 2 (garlic) extracted from garlic using the thermal rearrangement conditions.\[13\] The results are expressed as a mixture of (E)- and (Z)-ajoene. Both ajoene samples are effective QSIs as shown by their inhibition of the fluorescence in Figure 1, where a decrease in fluorescence is directly related to the downregulation of the QS gene lasB.

The samples show a very similar pattern of concentration-dependent inhibition. This is reiterated in the IC\(_{50}\) calculations where ajoene 2 (garlic) extracted from garlic had an IC\(_{50}\) value of 27.7 \(\mu\)M and synthetic ajoene 2 (synthetic) had an IC\(_{50}\) value of 28.5 \(\mu\)M. The IC\(_{50}\) values are comparable between the different origins of ajoene 2.

In conclusion, we have described an efficient total synthesis of ajoene from easily available starting materials. The simultaneous introduction of the allyl moiety and the sulfoxide group in the final step enabled the straightforward generation of the target molecule. Upscaling of the synthetic sequence was possible, leading to the synthesis of larger amounts of ajoene for the first time. Synthetic ajoene and ajoene derived from garlic were investigated regarding their efficiency as quorum sensing inhibitors.

**Experimental Section**

Vinyl disulfide 9a (0.140 g, 0.33 mmol) was dissolved in THF (3 mL) and cooled to 0°C under N\(_2\) and H\(_2\)O, (30\% w/w in H\(_2\)O, 0.075 mL, 0.66 mmol) was added dropwise. The mixture was stirred for 1 h at 0°C and then warmed to room temperature (2 h). Sat. aq. NaHCO\(_3\) (5 mL) was added, and the residue was extracted with EtOAc (2 \(\times\) 10 mL). The combined organic fractions were washed with brine (2 \(\times\) 10 mL) and dried over MgSO\(_4\). The solvent was removed under vacuum, and the resulting residue was purified by column chromatography to afford ajoene 2 (21 mg, 27\%, \(E/Z = 1:1.8\)) as a pale-yellow oil.

**Acknowledgements**

We thank Neem Biotech Ltd. and the EPSRC National Mass Spectrometry Facility, Swansea, for mass-spectrometric data.

**Conflict of interest**

The authors declare no conflict of interest.

**Keywords:** ajoene · allicin · garlic · organosulfur compounds · selenoxide elimination

**How to cite:** Angew. Chem. Int. Ed. 2018, 57, 12290–12293

Angew. Chem. 2018, 130, 12470–12473


[10] The scale-up reactions were performed by Onyx Scientific Ltd., UK.

