Synthesis of ustalic acid, an inhibitor of Na\textsuperscript{+},K\textsuperscript{+}-ATPase

Ichiro Hayakawa, Hidekazu Watanabe and Hideo Kigoshi *
Department of Chemistry, Graduate School of Pure and Applied Sciences, and Center for Tsukuba Advanced Research Alliance,
University of Tsukuba, Tennodai, Tsukuba 305-8571, Japan

Abstract— Ustalic acid, an inhibitor of Na\textsuperscript{+},K\textsuperscript{+}-ATPase isolated from a poisonous mushroom, was synthesized in 8 steps using the Suzuki–Miyaura coupling and oxidation of methylene acetal as key steps. © 2008 Elsevier Science. All rights reserved

1. Introduction

Ustalic acid (1) was isolated from a poisonous mushroom, Tricholoma ustale (Kakishimeji in Japanese) by Kawagishi et al. in 2002 (Fig. 1).\textsuperscript{1} Ustalic acid (1) inhibited Na\textsuperscript{+},K\textsuperscript{+}-ATPase; IC\textsubscript{50} values of ustalic acid (1) against the commercially available enzyme purified from porcine cerebral cortex and the crude enzyme from mouse intestinal mucosal cells were 5.2 and 0.77 mM, respectively. In 2006, Nishikawa et al. first achieved the total synthesis of ustalic acid dimethyl ester (2).\textsuperscript{2} We planned an efficient synthesis of ustalic acid (1), which will provide a practical supply for further biological studies. We report here the first synthesis of ustalic acid (1) in 8 steps using Suzuki–Miyaura coupling\textsuperscript{3} as a key step. Recently, Takahashi et al. have reported the total synthesis of a similar compound vialinin A, by a similar cross-coupling strategy.\textsuperscript{4}

2. Results and discussions

Our synthetic plan of ustalic acid (1) is shown in Scheme 1. Our synthetic route to ustalic acid (1) involved the Suzuki–Miyaura coupling\textsuperscript{3} at C-2–C-1’. We therefore synthesized organoboron compound 5.

\textsuperscript{4}Corresponding author: +81 29 853 4313; e-mail: kigoshi@chem.tsukuba.ac.jp.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Structures of ustalic acid (1) and derivative.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{scheme1.png}
\caption{Retrosynthetic analysis of ustalic acid (1).}
\end{figure}
Synthesis of the ustalic acid (1) started from commercially available sesame oil (Scheme 2). Sesame oil was transformed into catechol 7 and the hydroxyl group of 7 was then protected as an acetonide. The introduction of two boronic acid moieties into 8 was accomplished through a double lithiation by using n-BuLi and TMEDA at −78 °C and trapping with B(OMe)3 to give diboronic acid 9 and monoboronic acid 10.

Scheme 2. Synthesis of organoboronic acids. Reagents and conditions: a) PPTS, isopropenyl methyl ether, benzene, reflux, 96%, b) n-BuLi, TMEDA, B(OMe)3, THF, Et2O, −78 °C to rt; 9: 33%, 10: 24%.

We tried the Suzuki–Miyaura coupling 3 with diboronic acid 9 and iodobenzene (Scheme 3). The coupling reaction with Pd(PPh3)4 and Na2CO3 in dioxane afforded diphenyl compound 11 in one step, but the yield was low (25%). In contrast, the cross-coupling reaction of monoboronic acid 10 with iodobenzene proceeded by the same conditions to give monophenyl compound 12 in 71% yield. Compound 12 was converted into boronic acid 13 by lithiation followed by treatment with B(OMe)3. The coupling reaction of 13 with iodobenzene was subjected to the same conditions to give diphenyl compound 11.

Scheme 3. Study of Suzuki–Miyaura coupling. Reagents and conditions: a) PhI, Pd(PPh3)4, Na2CO3, dioxane, rt, 25%; b) PhI, Pd(PPh3)4, Na2CO3, dioxane, rt, 71%; c) n-BuLi, TMEDA, B(OMe)3, THF, Et2O, −78 °C to rt, 49%; d) PhI, Pd(PPh3)4, Na2CO3, dioxane, rt, 40%.

The investigation of removal of the acetonide group in 11 is summarized in Table 1. Acidic hydrolysis of 11 gave phlebiarubrone (3), an oxidative compound of catechol 14 (entry 1), which was isolated from the culture of the fungus Phlebia strigozonata. Synthetic phlebiarubrone (3) gave spectral data (1H NMR, 13C NMR, and HRMS) in full agreement with those of the natural one. Because catechol 14 readily accepted air oxidation, 14 was not isolated. Because of the irreproducible yield, we tried to synthesize ortho-quinone 3 by selective oxidation (entries 2–5). The reaction of 11 with DDQ gave only the undesired ortho-quinone 15 (entry 2). The oxidation by ammonium cerium(IV) nitrate (CAN) afforded the desired ortho-quinone 3 (12% yield) and the undesired ortho-quinone 15 (35% yield) (entry 3). The reactions at low temperature increased the selectivity of ortho-quinone 3 (entries 4 and 5). However, we could not satisfy the yield and selectivity in this transformation; therefore, we next tried oxidation of protected catechol by two methylene acetal groups.

Table 1. Study of the removal of the acetonide group in 11.
The hydroxyl groups of catechol 7 were protected by the second methylene acetal (Scheme 4). The bis-methylene acetal 16 was converted to diboronic acid 17 by double lithiation followed by treatment with B(O-i-Pr)3. Another borate reagent, B(OMe)3, was less effective in this case. Furthermore, the bis-methylene acetal 16 was converted to boronate 19 and pinacolborane 20 by sequential boronation and esterfication.

Next, we attempted a Suzuki–Miyaura coupling, as depicted in Table 2. A cross-coupling reaction between diboronic acid 17 and iodobenzene with Pd(PPh3)4 afforded monophenyl compound 22 and bis-methylene acetal 16 (entries 1 and 2). An attempt at a cross-coupling reaction of the diboronic acid 17 with PdCl2(PPh3)2 and Cs2CO3 in DMF at room temperature gave the desired diphenyl compound 21, but the yield was low (8%) (entry 3). The reaction at 90 °C afforded the desired diphenyl compound 21 in 30% yield along with monophenyl compound 22 (18%) and bis-methylene acetal 16 (4%) (entry 4). The cross-coupling reaction of the boronate 19 with PdCl2(PPh3)2 and Cs2CO3 in DMF afforded the desired diphenyl compound 21 in 43% yield (entry 5). Treatment of pinacolborane 20 under the same conditions gave the desired diphenyl compound 21 in 43% yield (entry 5). Treatment of pinacolborane 20 under the same conditions gave the desired diphenyl compound 21 in 43% yield (entry 5). Treatment of pinacolborane 20 under the same conditions gave the desired diphenyl compound 21 in 43% yield (entry 5). Treatment of pinacolborane 20 under the same conditions gave the desired diphenyl compound 21 in 43% yield (entry 5).

Since the diphenyl compound 21 has been synthesized in an available yield, we attempted oxidation of 21. Oxidation of 21 with CAN gave phlebiarubrone (3) in quantitative yield (Scheme 6). To convert phlebiarubrone (3) to ustalic acid dimethyl ester (2), we followed the procedure reported by Nishikawa et al. with a modification. Phlebiarubrone (3) was treated with Pb(OAc)4 (20 equiv) in MeOH and toluene in the presence of K2CO3 to give ustalic acid dimethyl ester (2). This modification increased the yield of the ustalic acid dimethyl ester (2) to 11%. Hydrolysis of the ustalic acid dimethyl ester (2) with 3 M KOH aq. in DMSO at room temperature for 1 day afforded the ustalic acid monomethyl ester (23). The monomethyl ester 23 was treated under the same conditions for 4 days to give ustalic acid (1) in 38% yield. The reaction at 70 °C for 39 h gave ustalic acid (1) in 23% yield. Synthetic ustalic acid (1) gave spectral data (1H NMR, 13C NMR, IR, and HRMS) in full agreement with those of the natural one, thus completing the total synthesis.
Table 2. Study of Suzuki–Miyaura coupling.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Catalyst</th>
<th>Solvent</th>
<th>Base</th>
<th>Temp.</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>Pd(PPh₃)₄</td>
<td>1,4-dioxane</td>
<td>Na₂CO₃ aq</td>
<td>rt</td>
<td>0% 16% 31%</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>PdCl₂(dppf)</td>
<td>DME</td>
<td>K₃PO₄ aq</td>
<td>60 °C</td>
<td>0% 43% 8%</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>PdCl₂(PPh₃)₂</td>
<td>DMF</td>
<td>Cs₂CO₃ aq</td>
<td>rt</td>
<td>8% 13% 11%</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>PdCl₂(PPh₃)₂</td>
<td>DMF</td>
<td>Cs₂CO₃ aq</td>
<td>90 °C</td>
<td>30% 18% 4%</td>
</tr>
<tr>
<td>5*</td>
<td>19</td>
<td>PdCl₂(PPh₃)₂</td>
<td>DMF</td>
<td>Cs₂CO₃</td>
<td>rt</td>
<td>43% 16% 32%</td>
</tr>
<tr>
<td>6*</td>
<td>20</td>
<td>PdCl₂(PPh₃)₂</td>
<td>DMF</td>
<td>Cs₂CO₃</td>
<td>90 °C</td>
<td>62% 3.2% 0%</td>
</tr>
</tbody>
</table>

* Isolated yields calculated from 16 (in 3 steps).

3. Conclusion

In summary, we achieved the first synthesis of ustalic acid (1) by using the Suzuki–Miyaura coupling as a key step. Further structure–activity relationship studies are now in progress.

4. Experimental

4.1 General methods.

¹H NMR spectra were recorded on a JEOL JNM-EX270 (270 MHz), or a Bruker AVANCE 500 (500 MHz) spectrometer. Chemical shifts for ¹H NMR are reported in parts per million (ppm) downfield from tetramethylsilane as the internal standard, and coupling constants are in hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad. ¹³C NMR spectra were recorded on a JEOL JNM-EX270 (67.8 MHz), or a Bruker AVANCE 500 (125 MHz) spectrometer. Chemical shifts for ¹³C NMR are reported in ppm, relative to the central line of a triplet at 77.0 ppm for deuteriochloroform. IR spectra were recorded on a JASCO FT/IR-300 instrument and are reported in wavenumbers (cm⁻¹). ESI mass spectra were recorded on a Applied Biosystems QStar/Pulsar i spectrometer. Elemental analyses were recorded on a Yanaco CHN CORDER MT-6. TLC analysis were conducted on E. Merck precoated silica gel 60 F₂₅₄ (0.25 mm layer thickness). Fuji Silysia silica gel BW-820 MH was used for column chromatography unless otherwise noted. Organic solvents for moisture-sensitive reactions were distilled from the following drying agents: THF, Et₂O, DME, and 1,4-dioxane (Na-benzophenone ketyl), benzene and toluene (Na). Anhydrous acetone, MeOH, CH₂Cl₂ and DMF were purchased from Kanto Chemical Co., Inc., or Wako Pure Chemical Industries, Ltd., and used without further drying. All moisture-sensitive reactions were performed under an atmosphere of argon or nitrogen, and the starting materials were azeotropically dried with benzene before use. All new compounds were determined to be >95% pure by ¹H NMR unless otherwise noted.

4.2 Bis-methylene acetal 16.

To a stirred solution of catechol 7 (1.02 g, 6.62 mmol) and Cs₂CO₃ (2.67 g, 8.19 mmol) in DMF (10 mL) was added CH₂Br₂ (0.71 mL, 9.93 mmol) at room temperature, and the mixture was stirred at 90 °C for 19 h. After cooling to room temperature, the mixture was diluted with Et₂O (30 mL), filtrated with Celite, and this Celite was rinsed with Et₂O (3×6 mL). The filtrate and rinse were washed with
H2O (3×10 mL), and the combined organic layer was extracted with Et2O (3×6 mL). The combined organic layers were washed with 1 M NaOH aq. (×3), H2O (×2), and brine (×1); dried (Na2SO4); and concentrated. The residual oil was purified by column chromatography on silica gel (15 g, n-hexane–EtOAc 30:1→10:1) to give bis-methylene acetal 16 (489 mg, 46%) as a white solid: colorless crystals; Mp 139-141 °C [(2.34 g, 7.18 mmol) at room temperature in a glove box. The mixture was stirred at 90 °C for 14 h under nitrogen flow and diluted with H2O (10 mL) at room temperature. The resultant mixture was stirred at room temperature for 17 h, diluted with 1 M HCl to pH 1, and the mixture was filtered with Celite. The filtrate and rinse were combined and this Celite was rinsed with CH2Cl2 (3×15 mL). The combined Et2O layers were washed with 1 M HCl (×2), H2O (×1), and brine (×1); dried (Na2SO4); and concentrated. The residual oil was purified by recrystallization from n-hexane–CH2Cl2 to give diphenyl compound 21 (21.7 mg, 2.8% in 3 steps; total 473 mg, 62% in 3 steps) and monophenyl compound 22 (18.7 mg, 3.2% in 3 steps) as colorless crystals, respectively: Diphenyl compound 21: Mp 204-205 °C (n-hexane–CH2Cl2); 1H NMR (270 MHz, CDCl3) δ 7.82-7.86 (m, 4H), 7.42-7.48 (m, 4H), 7.31-7.37 (m, 2H), 6.00 (s, 4H); 13C NMR (67.8 MHz, CDCl3) δ 139.0, 131.2, 128.8, 128.2, 127.8, 107.8, 100.8; Anal. Calcd. for C30H32NaO4 [M+Na]+ 453.2485; found 453.2492.

4.3 Diphenyl compound 21.

To a stirred solution of bis-methylene acetal 16 (400 mg, 2.41 mmol) in Et2O (10 mL) at 0 °C were added TMEDA (1.0 mL, 6.71 mmol) and n-BuLi (1.61 M solution in n-hexane, 4.5 mL, 7.25 mmol) under nitrogen flow, and the resultant mixture was stirred at 0 °C for 30 min. After cooling to –78 °C, B(O-i-Pr)3 (2.8 mL, 12.2 mmol) in Et2O (4.2 mL) was added, and the resultant mixture was stirred at 0 °C for 1 h. The mixture was stirred at room temperature for 17 h, diluted with 1 M HCl to pH 1, and extracted with CHCl3 (4×15 mL). The combined extracts were dried (Na2SO4) and concentrated to afford crude diboronic pinacol ester 17 (670 mg), which was used for the next reaction without further purification.

The crude diboronic acid 17 (670 mg), pinacol (2.26 g, 17.18 mmol) and MgSO4 (1.30 g, 10.8 mmol) were dissolved in CH2Cl2 (16 mL), and the resultant mixture was stirred at room temperature for 17.5 h. The mixture was filtered with Celite, and this Celite was rinsed with CH2Cl2 (3×5 mL). The filtrate and rinse were combined and this Celite was rinsed with CH2Cl2 (3×6 mL). The combined organic layers were washed with 1 M HCl (×2), H2O (×1), and brine (×1); dried (Na2SO4); and concentrated. The residual oil was purified by column chromatography on silica gel (15 g, n-hexane–EtOAc 30:1) to give bis-methylene acetal 16 (670 mg, quant.) as red crystals: Mp 139-141 °C [(18.7 mg, 3.2% in 3 steps) as colorless crystals, respectively: Diphenyl compound 21: Mp 204-205 °C (n-hexane–CH2Cl2); 1H NMR (270 MHz, CDCl3) δ 7.82-7.86 (m, 4H), 7.42-7.48 (m, 4H), 7.31-7.37 (m, 2H), 6.00 (s, 4H); 13C NMR (67.8 MHz, CDCl3) δ 139.0, 131.2, 128.8, 128.2, 127.8, 107.8, 100.8; Anal. Calcd. for C30H32NaO4 [M+Na]+ 453.2485; found 453.2492.

4.4 phlebiarubrone (3).

To a stirred solution of diphenyl compound 21 (410 mg, 1.28 mmol) in acetonitrile (97 mL) was added CAN (1.0 M solution in H2O, 3.9 mL, 3.90 mmol) at 0 °C, and the mixture was stirred at 0 °C for 3 min. The mixture was diluted with H2O (80 mL) and extracted with CHCl3 (3×15 mL). The combined extracts were dried (Na2SO4) and concentrated. The residual oil was purified by column chromatography on silica gel (10 g, n-hexane–EtOAc 10:1→4:1) to give red solid. The red solid was purified by recrystallization from n-hexane–CH2Cl2 to give phlebiarubrone (3) (389 mg, quant.) as red crystals: Mp 248-250 °C (n-hexane–CH2Cl2); 1H NMR (270 MHz, CDCl3) δ 7.60-7.65 (m, 4H), 7.34-7.48 (m, 6H), 6.13 (s, 2H); 13C NMR (67.8 MHz, CDCl3) δ 175.9, 156.1, 129.4, 128.7, 128.6, 128.1, 113.7, 102.7; HRESIMS m/z 327.0636, calcd for C19H14NaO4 [M+Na]+ 327.0635.

4.5 ustalic acid dimethyl ester (2).

Phlebiarubrone (3) (100 mg, 0.329 mmol), Pb(OAc)4 (2.92 g, 6.59 mmol), and K2CO3 (96.2 mg, 0.696 mmol) were dissolved in toluene (8.2 mL) and MeOH (5.8 mL). The resultant mixture was stirred at room temperature for 47 h, diluted with H2O (15 mL) and ethylene glycol (a few drops), and filtered with Celite. The filtrate was extracted with Et2O (4×15 mL). The combined extracts were washed with H2O, saturated aqueous NaHCO3, and H2O; dried (Na2SO4); and concentrated. The residual oil was purified by column chromatography on silica gel (8.0 g, n-hexane–EtOAc 5:1→2:1), preparative TLC (CH2Cl2), and preparative TLC (n-hexane–EtOAc 2:1) to give ustalic acid dimethyl ester (2) (13 mg, 11%) as a yellow solid. IR (film) 1718, 1637 cm–1; 1H NMR (270 MHz, CDCl3) δ 7.31-7.42 (m, 10H), 5.41 (s, 2H), 3.73 (s, 6H); 13C NMR (67.8 MHz, CDCl3) δ 167.5, 148.6, 134.4, 129.8, 127.9, 127.8, 115.2,
The ustalic acid dimethyl ester (2) (2.6 mg, 7.10 µmol) was treated with 3 M KOH aq.–DMSO (1:1, 0.45 mL) at room temperature for 24 h. The mixture was diluted with saturated NaH₂PO₄ to pH 3 and extracted with CHCl₃ (×4). The combined extracts were dried (Na₂SO₄) and concentrated to afford crude ustalic acid monomethyl ester (23) (2.3 mg), which was used for the next reaction without further purification.

The crude ustalic acid monomethyl ester (23) (2.3 mg) was treated with 3 M KOH aq.–DMSO (1:1, 0.45 mL) at room temperature for 4 days. The mixture was diluted with saturated NaH₂PO₄ to pH 3 and extracted with CHCl₃ (×4). The combined extracts were dried (Na₂SO₄) and concentrated to afford crude ustalic acid (1). The residual oil was purified by HPLC (Develosil ODS-HG-5 (250×20 mm), flow rate 5 mL/min; detection, UV 254 nm; solvent 50% MeOH/0.1% TFA) to give ustalic acid (1) (0.9 mg, 38%, retention time 91.2 min) as a white solid: IR (film) 1698, 1630 cm⁻¹; ¹H NMR (270 MHz, CDCl₃) δ 7.29-7.41 (m, 10H), 5.46 (s, 2H); ¹³C NMR (67.8 MHz, CDCl₃) δ 174.2, 149.2, 133.4, 129.9, 128.2, 127.9, 115.0, 96.3; HRESIMS m/z 361.0686, calcd for C₁₉H₁₄NaO₆ [M+Na]⁺ 361.0688.

Acknowledgments

This work was supported in part by Grants-in-Aid for Scientific Research on Priority Area (No. 16073204, “Creation of Biologically Functional Molecules”); and by the 21st COE program from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. We would like to thank Mr. Ikuo Iida (University of Tsukuba) for the elemental analyses.

References