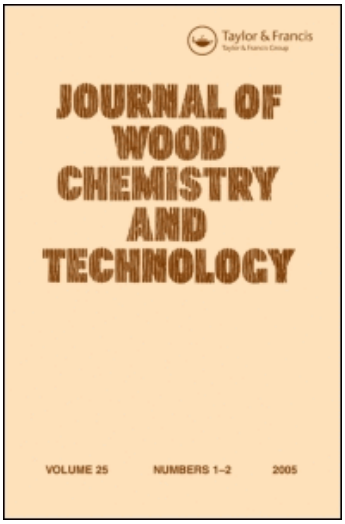

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Synthesis of Hydroxyphenylpropanoid β-D-glucosides

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Abstract: Dihydroconiferyl alcohol glucoside has been synthesized from cinnamic acid ethyl ester glucoside. Two reaction systems were investigated; one involving hydrogenation of the cinnamic acid ethyl ester glucoside intermediate followed by diisobutylaluminium hydride (DIBAL-H) reduction of the ester to the alcohol, and the other involving DIBAL-H reduction of the cinnamic acid ethyl ester to the alcohol (coniferin) followed by hydrogenation. Hydrogenation followed by DIBAL-H

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reduction led to higher dihydroconiferyl alcohol glucoside yields and avoided by-product formation associated with the hydrogenation of coniferin.

Keywords: Dihydroconiferyl alcohol glucoside, coniferin, synthesis, monolignol

INTRODUCTION

In recent years a mutant loblolly pine (Pinus taeda L.) was discovered deficient in cinnamyl alcohol dehydrogenase (CAD),[1] the enzyme catalyzing the conversion of 4-hydroxycinnamaldehydes to 4-hydroxycinnamyl alcohols, the monomers principally used in ligninification of plant cell walls.[2] During lignification, these monomers are converted to glucosides, transported to specific regions within the cell wall where they are converted to phenoxy radicals and undergo radical coupling reactions with other monolignol radicals or radicals that are part of the growing lignin polymer.[3,4] The end result is a very complex lignin macromolecule containing various interunit linkages.

In the CAD-deficient mutant pine the lignin content was only slightly reduced;[5] however, the lignin structure was dramatically modified. Lignins from CAD-deficient pine displayed unusually high levels of coniferaldehyde and dihydroconiferyl alcohol (DHCA).[2,6] Although the coniferaldehyde can be expected based on the nature of the mutation, DHCA is not normally associated with the lignin biosynthetic pathway, accounting for only 2–3 percent in normal pine. However, in the mutant lignin DHCA was found to contribute to as much as 30 percent of the monomeric component.[2,5,7] It is not completely clear as to the origin of DHCA. Ample evidence has been presented to indicate that it is formed as a monomer, and that monomer is incorporated into the lignin via the radical coupling process. It is not, however, known whether it arises from coniferyl alcohol or coniferaldehyde.

In an attempt to further elucidate the possible mechanism of DHCA incorporation into lignin we are studying the effect of monolignol and monolignol glucoside feeding experiments in cell culture. To accomplish this we need a method to produce the DHCA and DHCA-glucoside. Here we report a practical synthetic route to dihydroconiferyl alcohol β-D-glucoside (dihydroconiferin).

EXPERIMENTAL

All chemicals and solvents were purchased from Aldrich and used as received. ^{1}H NMR spectra were measured using a Bruker AVANCE-300 spectrometer at 40°C. Chemical shifts were referenced to tetramethyl silane (TMS; 0.0 ppm).

4-(β-D-glucopyranosyloxy)-3-methoxy-Benzaldehyde; Vanillin glucoside (**1**): Acetobromo-α-D-glucose, (10 g, 24.4 mmol)(95% TLC, Sigma®) and equimolar amount of vanillin (3.7 g, 24.4 mmol) (99%, Aldrich®) were stirred in 60 mL quinoline (98%, Sigma®) at 0°C. Silver (I) oxide (5.64 g, 24.4 mmol) was added portionwise over 10 min. The mixture was warmed to room temperature and stirred at moderate speed. After 1 h, 60 mL concentrated acetic acid (Fisher) was slowly added and the mixture was poured onto 1200 mL of water. The crushed product was filtered and dissolved in acetone. Silver oxide was removed by filtration and the filtrate was rotary evaporated yielding crude product in 84.8% yield (determined by NMR). It was then purified by two recrystallizations from ethanol yielding off-white, needle crystals in 75% yield (6.4 g), mp 196–198°C.

^{1}H NMR (300 MHz, $CDCl_3$): 2.08 (m, 12H), 3.86 (m, 1H), 3.91 (s, 3H), 4.25 (dd, 2H), 5.34 (m, 1H), 5.32 (m, 1H), 5.20 (m, 1H), 5.12 (d, 1H, $J_{1,2} = 7.5$ Hz), 7.22 (d, 1H), 7.42 (m, 2H), 9.91 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): 20.6 (OAc); 56.0 (CH_3O); 62.0 (C_6); 68.7 (C_4); 71.3 (C_2); 71.9 (C_5); 72.7 (C_3); 102.1 (C_1); 110.5 (C_2); 118.0 (C_5); 126.6 (C_6); 131.0 (C_1); 152.1 (C_3); 154.5 (C_4); 168.2–170.8 (OAc); 190.8 (C_α).

3-[3-methoxy-4-[(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)oxy]phenyl]-(E)-2-Propenoic acid-ethyl ester, Cinnamic acid ethyl ester glucoside (**2**): Compound **1** (5.4 g, 11.3 mmol) and monoethyl malonic acid (2.09 g, 15.5 mmol) were stirred in 30 mL pyridine (99.8%, Sigma-Aldrich®) and 0.5 mL piperidine (99.5%, redistilled, Sigma-Aldrich®). The solution was heated to 100°C and stirred for 1.5 h. The reaction flask was cooled to room temperature and 200 mL of distilled water was added followed by the addition of 50 mL 2.0 M hydrochloric acid. The product was filtered and recrystallized from ethanol. The final product (in the form of off-white powder) was obtained in 79.6% yield; mp 90–92°C.

^{1}H NMR (300 MHz, $CDCl_3$): 1.36 (t, 3H), 2.08 (m, 12H), 3.80 (m, 1H), 3.87 (s, 3H), 4.25 (m, 2H), 4.28 (q, 2H), 5.10 (d, 1H, $J_{1,2} = 7.8$ Hz), 5.18 (m, 1H), 5.30 (m, 2H), 6.35 (d, 1H, $J_{\alpha,\beta} = 15.5$ Hz),7.08 (m, 2H), 7.12 (d, 1H), 7.63 (d, 1H, $J_{\alpha,\beta} = 15.5$ Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 14.3 (OCH_2CH_3); 20.7 (OAc); 56.0 (CH_3O); 60.5 (OCH_2); 61.9 (C_6); 68.3 (C_4); 71.1 (C_2); 72.1 (C_3); 72.5 (C_5); 100.3 (C_1); 111.4 (C_2); 117.6 (C_β); 119.5 (C_5); 121.6 (C_6); 131.0 (C_1); 143.9 (C_β); 147.8 (C_4); 150.6 (C_3); 166.9 (C_γ); 169.4–170.2 (OAc).

4-(3-hydroxy-1-propenyl)-2-methoxyphenyl-β-D-Glucopyranoside Coniferin (**3**): Freshly dried glucoside (**2**) (3.9 g, 6.8 mmol) was dissolved in 120 mL dry toluene (99.8% Sigma-Aldrich®) and stirred under nitrogen atmosphere at 0°C for 15 min. DIBAL-H (85.5 mL, 85.5 mmol) (1.0 M solution in toluene, Aldrich®) was added dropwise over 45 min. The solution was then stirred at moderate speed for additional 1 h at 0°C and under N_2. The excess of DIBAL-H was quenched with 40 mL of cold ethanol added slowly to the

reaction flask. The mixture was stirred for another 30 min after which all solvents were removed by rotary evaporation, resulting in the formation of white gel. To the gel 200 mL of boiling water was added and the aluminium salts were removed by vacuum filtration. The residues were rinsed with additional 200 mL of H_2O. Filtrate was rotary evaporated yielding final product, which was recrystallized from water. The final yield was 75.1%.

^{1}H NMR (300 MHz, DMSO$_{-d6}$): 3.66 (m, 1H, H_5), 3.78 (s, 3H, OCH_3), 4.10 (t, 2H, H_γ), 4.51 (t, 1H, H_{6a}, $J_{6a,5}$ =5.7 Hz, $J_{6a,6b}$ =87 Hz), 4.80 (t, 1H, H_{6b}, $J_{6b,5}$ = 5.7 Hz, $J_{6b,6a}$ = 87 Hz), 4.89 (d, 1H, $J_{1,2}$ = 7.3 Hz), 5.00–5.17 (3H, $H_{2,3,4:}$ 5.00 (d, 1H, J = 4.5 Hz), 5.05 (d, 1H, J = 4.5 Hz), 5.19 (d, 1H, J = 5.2 Hz), 6.28 (dt, 1H, H_β, $J_{\beta,\alpha}$ = 15.7 Hz, $J_{\beta,\gamma}$ = 4.7 Hz), 6.47 (d, 1H, H_α, $J_{\alpha,\beta}$ = 15.7 Hz), 6.89 (dd, 1H, $H_{6'}$, $J_{6',5'}$ = 8.4 Hz, $J_{6',2'}$ = 1.4 Hz), 7.02 (d, 1H, $H_{5'}$, $J_{5',6'}$ = 8.4 Hz), 7.06 (d, 1H, $H_{2'}$, $J_{2',5'}$ = 1.4 Hz). ^{13}C NMR (75 MHz, DMSO$_{-d6}$): 56.1 (CH_3O); 61.1 (C_6); 62.1 (C_γ); 70.1 (C_4); 73.7 (C_2); 77.3 (C_5); 77.6 (C_3); 100.5 (C_1); 110.4 ($C_{2'}$); 115.6 ($C_{6'}$); 119.5 ($C_{5'}$); 128.9 (C_β); 129.5 (C_α); 131.5 ($C_{1'}$); 146.5 ($C_{4'}$); 149.5 ($C_{3'}$).

4-(3-hydroxypropyl)-2-methoxyphenyl-β-D-Glucopyranoside: **Dihydroconiferin** (**4**): Glucoside (**2**) (0.5 g, 0.9 mmol) was dissolved in 30 mL CH_2Cl_2. 5mol% Platinum on activated Carbon (Pt content 5%, Aldrich®) (0.173 g, 0.04 mmol) was added and the reaction flask was flushed with N_2 for 15 min, then with H_2 for 30 min. Upon flushing, the reaction mixture was kept under 1 atm H_2 for 2 h. The catalyst was removed by filtration and the solvent was rotary evaporated yielding yellow oil, which upon standing gave silver crystals of saturated glucoside.

^{1}H NMR (300 MHz, $CDCl_3$): 1.26 (t, 3H), 2.07 (m, 12H), 2.61 (t, 2H), 2.92 (t, 2H), 3.77 (m, 1H), 3.82 (s, 3H), 4.15 (q, 2H), 4.24 (m, 2H), 4.94 (d, 1H, $J_{1,2}$ = 7.9 Hz), 5.18 (m, 1H), 5.30 (m, 2H), 6.75 (m, 1H), 6.80 (s, 1H), 7.05 (d, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) 14.2 (OCH_2CH_3); 20.6 (OAc); 30.7 (C_α); 36.0 (C_β); 56.1 (CH_3O); 60.5 (OCH_2) 62.0 (C_6); 68.5 (C_4); 71.3 (C_2); 71.9 (C_5); 72.7 (C_3); 101.0 (C_1); 112.9 ($C_{2'}$); 115.9 ($C_{5'}$); 120.6 ($C_{6'}$); 137.4 ($C_{1'}$); 144.5 ($C_{4'}$); 150.6 ($C_{3'}$); 169.4–170.6 (OAc); 172.8 (C_γ).

The product was then reduced with DIBAL-H in the same procedure that was used for the preparation of (**3**). The final yield of **4** was 88.3%; mp 117–119°C.

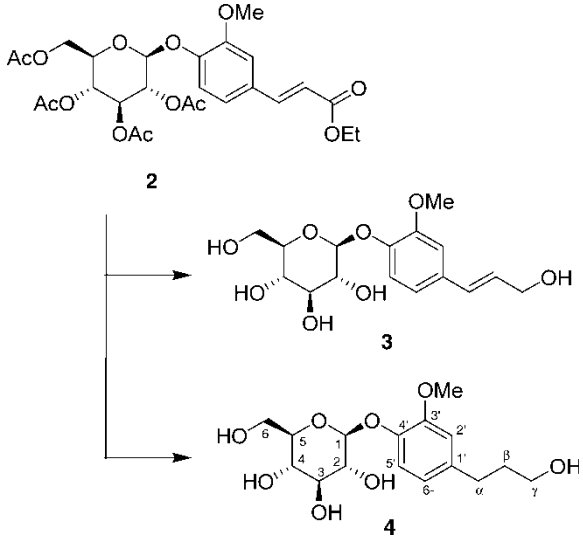
^{1}H NMR (300 MHz, DMSO$_{d6}$): 1.69 (m, 2H, H_β, $J_{\beta,\alpha}$ = 7.3 Hz, $J_{\beta,\gamma}$ = 6.6 Hz), 2.54 (t, 2H, H_α, $J_{\alpha,\beta}$ = 7.3 Hz), 3.24 (t, 2H, H_γ, $J_{\beta,\gamma}$ = 6.6 Hz,), 3.65 (m, 1H, H_5, $J_{5,6a}$ = 5.5 Hz, $J_{5,4}$ = 4.4 Hz), 3.74 (s, 3H, OCH_3), 4.48 (t, 1H, H_{6a}, $J_{6a,5}$ = 5.5 Hz, $J_{6a,6b}$ = 15.4 Hz), 4.53 (t, 1H, H_{6b}, $J_{6b,5}$ = 5.5 Hz, $J_{6b,6a}$ = 15.4 Hz), 4.83 (d, 1H, H_1, $J_{1,2}$ = 7.0 Hz), 5.00–5.17 (3H, $H_{2,3,4;}$ 5.00 (d, 1H, J = 4.9 Hz), 5.05 (d, 1H, J = 3.5 Hz), 5.17 (d, 1H, J = 4.4 Hz), 6.68 (dd, 1H, $H_{6'}$, $J_{6',5'}$ = 8.2 Hz; $J_{6',2'}$ = 1.5 Hz), 6.80 (d, 1H, $H_{2'}$, $J_{2',6'}$ = 1.5 Hz), 6.97 (d,1H, $H_{5'}$, $J_{5',6'}$ = 8.2 Hz). ^{13}C NMR (75 MHz, DMSO$_{d6}$): 31.7 (C_α); 34.9 (C_β); 56.1 (CH_3O); 60.6 (C_6); 61.2 (C_γ); 70.2 (C_4); 73.7 (C_2); 77.3 (C_3); 77.5 (C_5); 100.8 (C_1); 113.3 ($C_{2'}$); 115.9 ($C_{5'}$); 120.6 ($C_{6'}$); 136.4 ($C_{1'}$); 145.1 ($C_{4'}$); 149.3 ($C_{3'}$).

RESULTS AND DISCUSSION

The synthesis of the various glucosides was conducted based on modifications of reported synthesis.[8–10] However, to our knowledge no synthetic route to dihydroconiferyl alcohol β-D-glucoside has been reported. As shown in Scheme 1 the essential intermediate in our synthetic route is the cinnamic acid ethyl ester glucoside (**2**), which can be converted to coniferin and dihydroconiferin.

The cinnamic acid ethyl ester glucoside (**2**) was obtained as per Figure 1 by reacting α-bromo-D-acetoglucose with vanillin using quinoline and silver (I) oxide. ^{1}H NMR analysis confirmed the formation of only the β-anomer (α-anomeric proton, $J = 7.5$ Hz, *vs.* $J = 4.2$ Hz for the β- anomeric proton in the α-bromo-D-acetoglucose). Vanillin glucoside (**1**) was converted to the cinnamic acid ethyl ester (**2**) using monethyl malonic acid and piperidine.[11] Previous methods utilized malonic acid to obtain the coniferyl alcohol glucoside; however, the yields were poor as it involved creating and purifying the acyl chloride to further reduce it to the alcohol. This was not an attractive route to follow because the product would not allow access to all the target compounds.

Reduction of the cinnamic acid ethyl ester (**2**) with DIBAL-H produced the target glucoside, coniferin (**3**) in good yields. However, hydrogenation of the double bond in **3** to produce the corresponding dihydroconiferyl alcohol glucoside (**4**) proved to be problematic. As shown in Figure 2, a reductive elimination product **5** was also produced. NMR analysis of the crude reaction revealed



Scheme 1. Synthetic route for the production of coniferyl alcohol (coniferin) and dihydroconiferyl alcohol (dihydroconiferin) glucosides.

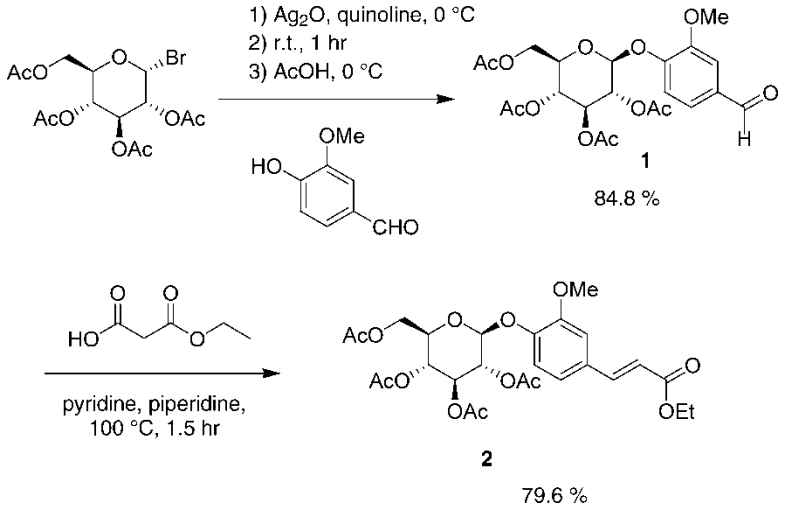


Figure 1. Synthesis of cinnamic acid ethyl ester (**2**).

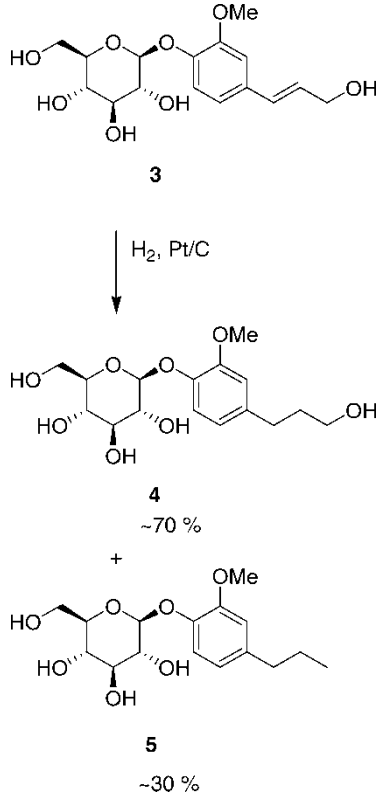


Figure 2. Hydrogenation of coniferin (**3**) to dihydroconiferin (**4**).

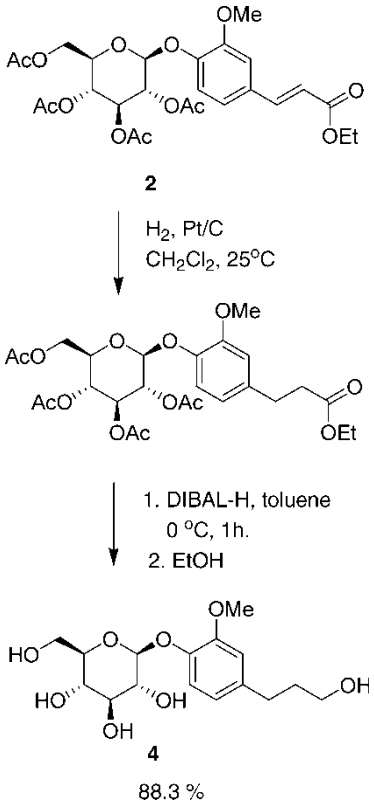


Figure 3. Hydrogenation and reduction of cinnamic acid ethyl ester glucoside (**2**) to dihydroconiferyl alcohol glucoside (**4**).

approximately 30% of **3** was converted to the propylphenol derivative (**5**). Purification of **4** in the presence of **5** was found to be extremely difficult, owing to the similar R_f of **4** and **5**, which co-eluded in the various cosolvents investigated.

To eliminate by-product formation and improve dihydroconiferyl alcohol glucoside (**4**) yields, the cinnamic acid ethyl ester glucoside (**2**) was directly hydrogenated and the saturated ester intermediate reduced with DIBAL-H (Figure 3). No by-products were detected, and dihydroconiferyl alcohol glucoside (**4**) was obtained in $\sim$ 88% yield; corresponding to ~60% total yield based on vanillin and α-bromo-D-acetoglucose.

CONCLUSIONS

This article reported a simple synthetic scheme to produce dihydroconiferyl alcohol glucoside. The process involves the hydrogenation of the cinnamic

acid ethyl ester glucoside intermediate followed by DIBAL-H reduction of the ester to the alcohol. This process produces higher yields and avoids by-product formation associated with hydrogenation of coniferin.

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