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First synthesis of (1,2-$^{13}C_2$)-monolignol glucosides

Vickram Beejmohun,[a,b] Eric Grand,[a] François Mesnard,[b] Marc-André Fliniaux[b] and José Kovensky[a,*]

[a]*Laboratoire des Glucides CNRS FRE 2779, Faculté des Sciences, Université de Picardie 'Jules Verne', 33 rue Saint-Leu, 80039 Amiens, France*

[b]*Laboratoire de Phytotechnologie, Faculté de Pharmacie, Université de Picardie 'Jules Verne', 1 rue des Louvels, 80037 Amiens, France*

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Abstract—The monolignol glucosides (1,2-$^{13}C_2$)-*p*-glucocoumaryl alcohol, (1,2-$^{13}C_2$)-coniferin and (1,2-$^{13}C_2$)-syringin, and the glucosides of (1,2-$^{13}C_2$)-*p*-coumaric, (1,2-$^{13}C_2$)-ferulic and (1,2-$^{13}C_2$)-sinapic acids were synthesized by condensation of the corresponding aldehydes with (1,2,3-$^{13}C_3$)-malonic acid. The free acids were converted to the acyl chlorides prior to their reduction to alcohols.

Lignans are widely distributed plant metabolites associated with a range of biological activities. Podophylotoxin is used as an antiviral agent in the treatment of genital warts[1] and secoisolariciresinol diglucoside is a natural cancer chemopreventive agent effective against the onset of breast, prostate and colon cancers.[2] The biosynthetic pathway of lignans is not completely elucidated, as it is specific to each species of plants.[3] Most of the present knowledge about the conversion of monolignols to their dimers lignans and the formation of cell wall lignins in plants comes from incorporation experiments using specifically labelled precursors or substrates.[4,5]

Monolignol glucosides (*p*-glucocoumaryl alcohol, coniferin, syringin) are considered to be the storage or excretion form of monolignols.[6] The synthesis of monolignol glucosides enriched with ^{13}C at specific positions has been used to prove their effective incorporation in lignin.[7] However, to obtain detailed information about the biosynthetic route of lignans in vivo, the synthesis of multiple labelled precursors becomes necessary. The presence of two consecutive ^{13}C atoms in monolignol molecules allows to follow their conversion to dimers due to the characteristic signals observed in the NMR spectra of intermediates involved.

Only one synthesis of (1,2-$^{13}C_2$) coniferyl alcohol has been previously reported.[8] Therefore, we decided to prepare the three monolignol glucosides, that should be easily incorporated by plant cells. Glucosides of monolignols are involved in the supply of monolignols before polymerization.[9] Moreover, the carbohydrate moiety should make the substrate less toxic for in vitro cultured cells.

In this letter, we report on the synthesis of the labelled monolignol glucosides (1,2-$^{13}C_2$)-*p*-glucocoumaryl alcohol, (1,2-$^{13}C_2$)-coniferin and (1,2-$^{13}C_2$)-syringin and their precursors, the glucosides of (1,2-$^{13}C_2$)-*p*-coumaric, (1,2-$^{13}C_2$)-ferulic and (1,2-$^{13}C_2$)-sinapic acids.

The synthetic strategy follows in part the previously described preparation of unlabelled compounds[10–13] and is shown in Scheme 1. Conversion of *p*-hydroxybenzaldehyde (**1a**), vanillin (**1b**) and syringaldehyde (**1c**) to their corresponding β-glucosides was performed with tetra-*O*-acetyl-α-D-glucopyranosyl bromide and silver oxide in quinoline to give **2a**, **2b** and **2c** in 65%, 73% and 75% yields, respectively. The β-configuration of the glycosidic bond was confirmed by NMR ($J_{1',2'}$ = 7.8 Hz). Further attempts to substitute the high boiling point solvent by pyridine or triethylamine–$CaCO_3$ were unsatisfactory.

The introduction of the double ^{13}C labelling was accomplished by condensation of **2a**–**c** with readily available (1,2,3-$^{13}C_3$)-malonic acid leading, after decarboxylation,

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* Corresponding author. Tel.: +33 3 22 82 75 67; fax: +33 3 22 82 75 68; e-mail: jose.kovensky@u-picardie.fr

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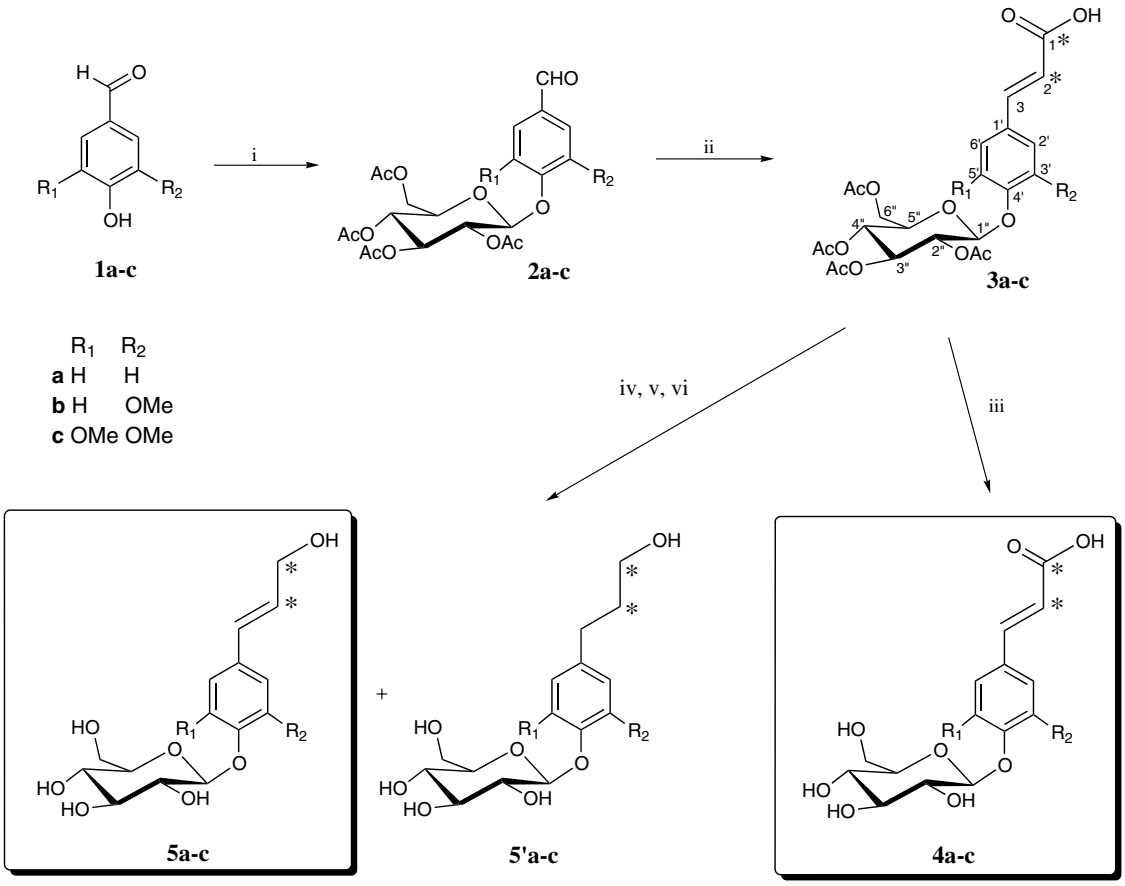

Scheme 1. Reagents and conditions: (i) tetra-*O*-acetyl-α-D-glucopyranosyl bromide, Ag_2O, quinoline, rt, 2 h; (ii) $^{13}CH_2(^{13}COOH)_2$, pyridine, piperidine, 1 h at 55 °C and 2 h at 85 °C; (iii) MeONa, MeOH, rt, overnight; (iv) $(COCl)_2$, CH_2Cl_2, rt, overnight; (v) $NaBH_4$, THF, rt, 4 h; (vi) NH_3, MeOH, rt, overnight.

to compounds **3a**–**c** in 70% yield. The typical pattern of consecutive labelling was observed in the ^{13}C NMR spectra. For example, in the spectrum of compound **3b** in $CDCl_3$ the two doublets appear at δ 171.3 (C-1) and δ 116.1 (C-2) with a $J_{1,2} = 74$ Hz.

Since the free acids are also interesting substrates for biosynthetic studies, the deprotection of the sugar moiety was performed in MeONa–methanol. After purification by flash chromatography on silica gel the acids **4a**, **4b** and **4c** were obtained as white solids.[14]

The synthesis of the labelled monolignols from the corresponding protected acids **3a**–**c** can be performed by two different routes: through esters or acyl chlorides derivatives. Both were explored using **3b** as model compound. First, the methyl ester was prepared using $KHCO_3$–MeI–TBAI in excellent yields (93–97%). Different reducing conditions were tested. $LiAlH_4$–$AlCl_3$ lead to low yields of the reduction product accompanied by degradation by-products. On the other hand, $LiBH_4$ at 60 °C or $LiAlH_4$ at room temperature allowed to accomplish the reduction of the carboxyl group in 63% and 90% yields, respectively. However, after deacetylation, NMR and mass spectra showed that the isolated product was in fact a mixture of the expected allylic alcohol **5b** and about 30–50% of a product resulting from the reduction of the 2,3-double bond (compound **5′b**). Changes in reagent and/or substrate concentration, solvent and temperature were unsuccessful to increase the **5b**/**5′b** ratio and gave no reproductible yields.

Therefore, we decided to change to the acyl chloride route. The reaction of **3b** with oxalyl chloride at room temperature gave the corresponding acyl chloride. Partial degradation was observed when the acyl chloride was prepared with thionyl chloride instead of oxalyl chloride.

The reduction of the acyl chloride with $NaBH_4$ is affected by the solvent employed. In diethyl ether, the reaction did not go to completion, while the use of THF at room temperature lead smoothly, after deacetylation, to the desired alcohol **5b**, keeping the level of the reduced double bond by-product **5′b** around 5%.

Therefore, the sequence acid–acyl chloride–reduction–deacetylation was applied to the three precursors **3a**, **3b** and **3c**, leading to the expected alcohols **5a**,**b** and **5c**, in 60%, 68% and 70% yields, respectively. The ^{13}C NMR spectra of the final products[15] showed the expected two doublets at δ 130.05 and 61.56 ($J_{1,2} = 47$ Hz).

In summary, the monolignol glucosides (1,2-$^{13}C_2$)-*p*-glucocoumaryl alcohol, (1,2-$^{13}C_2$)-coniferin and (1,2-$^{13}C_2$)-syringin and their acid precursors have been prepared for the first time.

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14. All new compounds were characterized by spectroscopical methods. Analytical data for compound **4c**: mp 253–254 °C; $[\alpha]_D$ −13 (*c* 0.04, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD): δ 7.35 (ddd, 1H, $J_{2,3}$ = 15.9, $J_{3,C\text{-}1}$ = 6.0, $J_{3,C\text{-}2}$ = 2.5 Hz, H-3), 6.88 (s, 2H, H-2′, H-6′), 6.48 (ddd, 1H, $J_{2,C\text{-}1}$ = 2.7, $J_{2,C\text{-}2}$ = 155.6 Hz, H-2), 4.93 (d, 1H, $J_{1'',2''}$ = 7.3 Hz, H-1″), 3.88 (s, 6H, 2CH_3O–), 3.79 (dd, 1H, $J_{5'',6''a}$ = 2.5, $J_{6''a,6''b}$ = 12.0 Hz, H-6″a), 3.68 (dd, 1H, $J_{5'',6''b}$ = 4.9 Hz, H-6″b), 3.50–3.40 (m, 3H, H-2″, H-3″, H-4″), 3.23 (ddd, 1H, $J_{4'',5''}$ = 7.3 Hz, H-5″). ^{13}C NMR δ 175.33 (d, $J_{1,2}$ = 67 Hz, C-1), 126.06 (d, C-2). ESMS (+Na): Calcd for $^{12}C_{15}{}^{13}C_2H_{22}O_{10}Na$: *m/z* 411.1178. Found: 411.1189.
15. Analytical data for compound **5c**: syrup; $[\alpha]_D$ −2 (*c* 0.07, CH_3OH); ^{1}H NMR (300 MHz, CD_3OD): δ 6.75 (s, 2H, H-2′, H-6′), 6.55 (ddd, 1H, $J_{2,3}$ = 16.0, $J_{3,C\text{-}1}$ = 6.0 Hz, $J_{3,C\text{-}2}$ = 3.0 Hz, H-3), 6.11 (dddt, 1H, $J_{1,2}$ = 4.0, $J_{2,C\text{-}1}$ = 2.5, $J_{2,C\text{-}2}$ = 154.0 Hz, H-2), 4.90 (d, 1H, $J_{1'',2''}$=7.3 Hz, H-1″), 4.22 (br dt, 2H, $J_{1,C\text{-}1}$ = 142.0, $J_{1,2}$ = $J_{1,C\text{-}2}$ = 4.3 Hz, H-1), 3.83 (s, 6H, 2CH_3O–), 3.78 (dd, 1H, $J_{5'',6''a}$ = 2.5, $J_{6''a,6''b}$ = 12.0 Hz, H-6″a), 3.68 (dd, 1H, $J_{5'',6''b}$ = 5.1 Hz, H-6″b), 3.45–3.35 (m, 3H, H-2″, H-3″, H-4″), 3.20 (m, 1H, H-5″). ^{13}C NMR δ 130.05 (d, $J_{1,2}$ = 47 Hz, C-2), 61.56 (d, C-1). ESMS (+Na): Calcd for $^{12}C_{15}{}^{13}C_2H_{24}O_9Na$: *m/z* 397.1385. Found: 397.1394.