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Short Note

New Facile Syntheses of Monolignol Glucosides; *p*- Glucocoumaryl Alcohol, Coniferin and Syrigin

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Introduction

The monolignols glucosides *p*-glucocoumaryl alcohol, coniferin and syringin, have been found in cambial sap of gymnospermous trees (Freudenberg and Harkin 1963) and some species of angiospermous trees (Terazawa *et al.* 1984). Coniferin has been shown to bean effective precursor of lignin biosynthesis in pine (Freudenberg *et al.* 1955). When three kinds of monolignol glucosides labeled with ^{14}C or ^{3}H were administered to growing stems of both gymnosperms and angiosperms, including monocotyledons, they were effectively incorporated into cell wall lignins in a biochemically regulated manner (Terashima *et al.* 1993). When monolignols glucosides enriched with ^{13}C at specific side-chain carbon were fed to plants, lignin was shown to be ^{13}C enriched at the corresponding positions (Terashima *et al.* 1991). A hypothetical mechanism for formation of lignified cell wall has been proposed in which monolignols glucosides play an important role as precursors of lignin biosynthesis (Terashima *et al.* 1995, 1996). Using this theory as a basis, a new type of synthetic guaiacyl lignin was prepared from coniferin that has a closer resemblance to natural lignin than do other synthetic lignins prepared from coniferyl alcohol by conventional procedures (Terashima *et al.* 1995, 1996).

In previous work, labeled or unlabeled monolignols glucosides employed as lignin precursors were synthesized mainly on the basis of procedures described by Kratzl and Billek (1953a, 1953b, 1954) and Fuchs (1955) as shown in Figure 1. First, the aromatic aldehydes *p*-hydroxy benzaldehyde, vanillin and syringaldehyde (Ia, Ib and Ic) were converted to their tetra-O-acetyl-ß- D-glucosides (IIIa, IIIb and IIIc), which were then condensed with malonic acid to give tetra-O-acetyl-ß-D-glucosides of corresponding *p*-coumaric, ferulic and sinapic acids (IVa, IVb and IVc) (Kratzl and Billek 1953). The acids were converted to corresponding acid chlorides (Va, Vb and Vc) by treatment with thionyl chloride, and the acid chlorides were reduced with sodium trimethoxyborohydride to give the corresponding alcohols (VIa, VIb and VIc) (Fuchs 1955). Deacetylation with sodium methoxide gave monolignols glucosides (VIIa, VIIb and VIIC) in 60–80% of theoretical yield.

More recently, Teutonico *et al.* (1991) prepared the glucoside of ß-5-dehydrodiconiferyl alcohol from the corresponding tetra-O-acetyl-ß-D-glucoside of ß-5-dehydrodiferulic acid dimethyl ester by reduction and deacetylation with isopropyl aluminum hydride. Quideau and Ralph (1992) also showed that diisobutylaluminum hydride (DIBAL-H) is useful for reduction of ethyl esters of *p*-coumaric, ferulic and sinapic acids to give corresponding monolignols in good yield.

These new developments have inspired us to develop a new procedure for synthesizing the monolignols glucosides *p*-glucocoumaryl alcohol, coniferin and syringin, (VIIa, VIIb and VIIc) from the corresponding acetylglucocinnamic acid esters (VIIIa, VIIIb and VIIIc) in good yield. Reduction of the esters to alcohols and removal of the protecting group (deacetylation) were achieved in one step (Fig. 2).

Experimental

NMR spectra

NMR spectra were obtained with Bruker WM-250 and AMX-360 instruments. Samples consisted of 25 mg compound dissolved in 0.5 ml d_6-DMSO, with TMS as an internal standard. Standard

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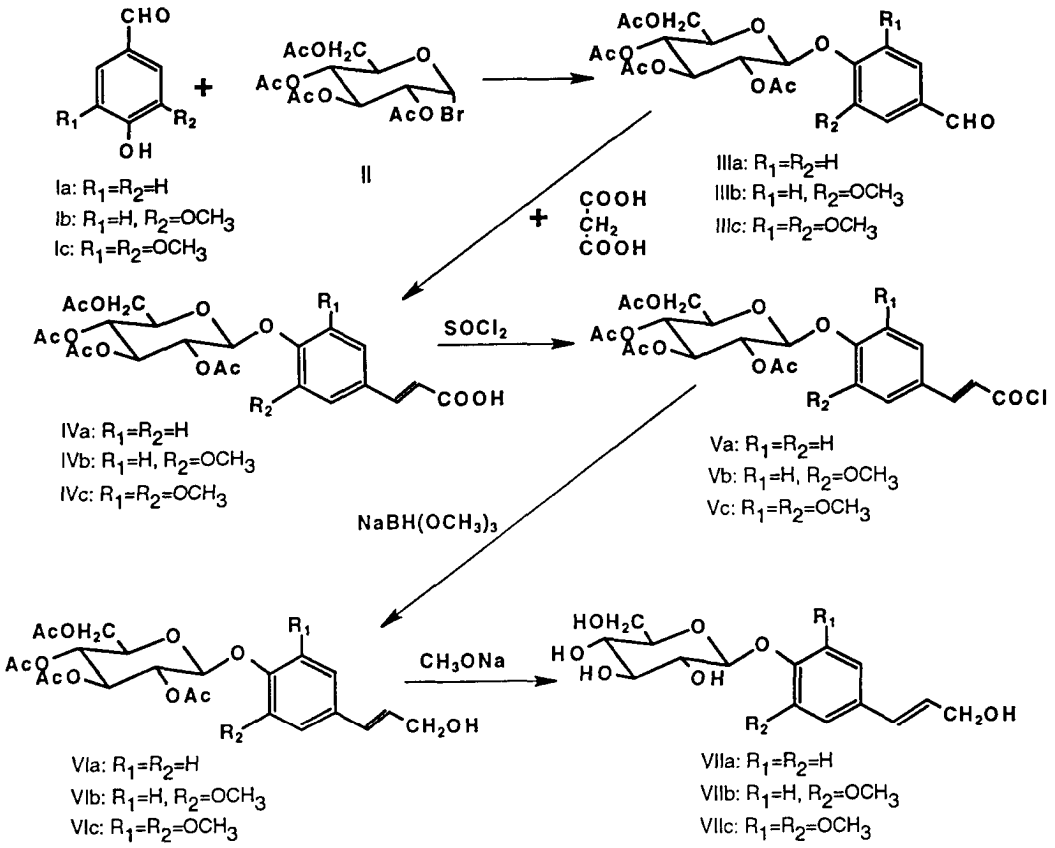

Fig. 1. Conventional syntheses of monolignol glucosides.

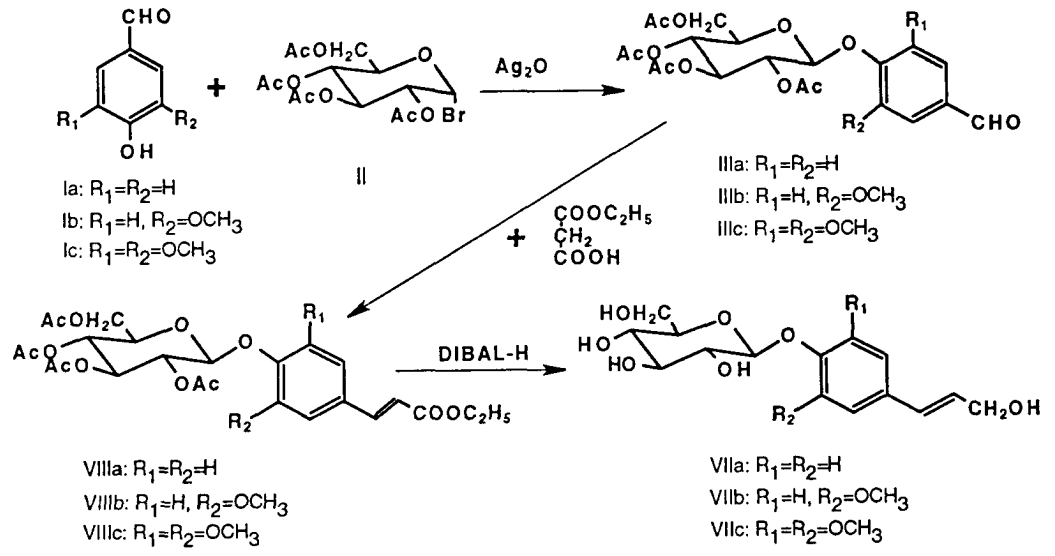

Fig. 2. New syntheses of monolignol glusosides.

Bruker pulse programs were used for HMQC, HMBC and COSY two dimensional spectra. Fully authenticated chemical shifts assignments were made for coniferin (VIIb) and the ethylferulate glocoside (VIIIb). Chemical shifts for the other compounds were assigned by inference, and restricted acquisition XHCORR experiments were used to confirm assignments of close chemical shifts.

Mass spectra

Mass spectra were obtained with a Finnigan Mass Spectrometer model 4500 by chemical ionization in ammonia at 70eV. MS of all nine compounds (IIIa, IIIb, IIIc, VIIIa, VIIIb, VIIIc, VIIa, VIIb, VIIc) contained parent peaks corresponding to M + NH_4^+.

4-O-[Tetra-O-ocetyl-ß-D-glucopyranosyl]-bellzaldellyde (IIIa)

p-Hydroxy benzaldehyde (Ia, 3.66g, 30mMol, Aldrich Chemical Co., Milwaukee, Wisconsin, U.S.A.), and tetra-O-acetyl-α-D-glucopyranosylbromide (II, 12.34g, 30mMol, Aldrich Chemical Co.) were dissolved in quinoline (30 ml, Aldrich Chemical Co.), and silver oxide (3.95 g, 18mMol, Aldrich Chemical Co.) was added portionwise with vigorous stirring. After the first exothermic reaction ended, stirring was continued for a further 20min. Acetic acid (20ml. Aldrich Chemical Co.) was added, and the whole mixture was poured into water (1000 ml). The fine precipitate was collected by filtration through a thick mat of pulp prepared from defibrated filter paper. After washing with water, the residue was extracted three times with hot ethanol (200 ml X 3). Concentration of the combined ethanol solution gave crystals. Recrystallization from ethanol afforded pure compound IIIa (8.40g, 61% of theoretical yield); mp. 142.5–144°C (lit. 144–145°C, Mauthner 1912). MS of molecular ion, m/z (M + NH_4^+) = 470.

4-O-[Tetra-O-acetyl- ß-D-gluco]-vanillin (IIIb) and 4-O-[tetra-O-acetyl-ß-D-gluco]-syringaldehyde (IIIc)

Using the procedure described for IIIa, compounds IIIb and IIIc were prepared in yields of 75% and 80% respectively; mp. 141–142°C (lit. 143–144°C, Fischer and Raske 1909), m/z (M + NH_4^+) = 500 (IIIb), mp. 157–159°C (lit. 156–159°C, Kratzl and Billek 1954), m/z (M + NH_4^+) = 530 (IIIc).

4-O-[Terra-O-acetyl-ß-D-gluco]-coumaric acid ethyl ester (VIIIa)

A mixture of compound IIIa (4.52g, 10mMol), monoethyl malonate (1.45g, 11mMol), prepared by the procedure described by Freudenberg and Hübner (1952), pyridine (4ml) and piperidine (0.15ml) was heated at 100°C for 1.5h. The mixture was poured into water (200ml) with stirring. The precipitate was collected by filtration and recrystallized with ethanol to give compound VIIIa (4.96g, 95%); mp. 141-142.5°C, m/z(M+NH_4^+)=540.

Table 1. ^{1}H NMR shifts of monolignol glucosides and intermediates in their syntheses

	III A	III B	III C	VIII A	VIII B	VIII C	VII A	VII B	VII C
α	9.92 s	9.90 s	9.90 s	7.62 d (16.0)	7.60 d (16.0)	7.59 d (15.9)	6.49 d (16.0)	6.47 d (15.9)	6.47 d (15.9)
β	–	–	–	6.55 d (16.0)	6.63 d (16.0)	6.70 d (15.9)	6.24 dt (15.9, 5.2)	6.28 dt (15.9, 5.0)	6.63 dt (15.9, 4.7)
γ'	–	–	–	–	–	–	4.09 dd (5.2)	4.10 dd (5.3)	4.11 dd (5.0)
γ'	–	–	–	–	–	–	4.09 dd (5.2)	4.10 dd (5.3)	–
2	7.92 d (8.7)	7.49 d (1.8)	7.27 s	7.72 d (8.7)	7.42 dd (1.6)	7.10 s	7.35 d (8.7)	7.06 d (1.8)	6.73 s
3	7.19 d (8.7)	–	–	7.03 d (8.7)	–	–	6.98 d (8.7)	–	–
5	7.19 d (8.7)	7.30 d (8.3)	–	7.03 d (8.7)	7.12 d (8.4)	–	6.98 d (8.7)	7.02 d (8.4)	–
6	7.92 d (8.7)	7.57 dd (8.3, 1.8)	7.27 s	7.72 d (8.7)	7.29 dd (8.4, 1.6)	7.10 s	7.35 d (8.7)	6.89 dd (8.4, 1.8)	6.73 s
1'	5.78 d (7.9)	5.63 d (7.9)	5.36 d (6.9)	5.65 d (7.9)	5.46 d (7.9)	5.23 d (7.8)	4.84 d (7.3)	4.89 d (7.3)	4.91 d (4.8)
2'	5.12 dd (9.7, 7.9)	5.11 dd (9.7, 7.9)	5.02 dd (7.7, 9.5)	5.08 dd (9.6, 8.0)	5.07 dd (9.7, 8.1)	5.03–4.94 m	3.36–3.14 nr	3.34–3.18 nr	3.18–3.04 nr
3'	5.44 dd (9.5)	5.42 dd (9.6)	5.34 dd (9.4)	5.42 dd (9.5)	5.39 dd (9.7)	5.32 t (9.5)	3.36–3.14 nr	3.34–3.18 nr	3.18–3.04 nr
4'	5.04 dd (9.7)	5.03 dd (9.6)	5.00 dd (9.5)	5.01 dd (9.6)	5.00 dd (9.7, 8.1)	5.03–4.94 m	3.36–3.14 nr	3.34–3.18 nr	3.18–3.04 nr
5'	4.35–4.06 nr	4.27–4.07 nr	4.20–3.74 nr	4.29–4.06 nr	4.23–4.05 nr	4.15–3.88 nr	3.36–3.14 nr	3.34–3.18 nr	3.18–3.04 nr
6a'	4.35–4.06 nr	4.27–4.07 nr	4.20–3.74 nr	4.29–4.06 nr	4.23–4.05 nr	4.15–3.88 nr	3.46 m (11.7, 6.0)	3.46 m	3.40 m
6b'	4.35–4.06 nr	4.27–4.07 nr	4.20–3.74 nr	4.29–4.06 nr	4.23–4.05 nr	4.15–3.88 nr	3.70 ddd (11.7)	3.67 ddd	3.59 ddd
6'OH	–	–	–	–	–	–	4.58 t (5.7)	4.54 t (5.7)	4.29 t (5.4)
γOH	–	–	–	–	–	–	4.83 t (5.4)	4.83 t (5.6)	4.85 t (5.5)
CH_2	–	–	–	4.18 q (7.0)	4.23–4.16 nr	4.19 q (7.1)	–	–	–
CH_3	–	–	–	1.26 t (7.0)	1.26 t (7.0)	1.26 t (7.0)	–	–	–
OMe	–	3.84 s	3.85 s	–	3.80 s	3.80 s	–	3.78 s	3.77 s
Ac Me's	2.02–1.98	2.02–1.98	1.98–1.96	2.02–1.98	2.02–1.97	1.98–1.96	–	–	–

nr = not resolved

Table 2. ^{13}C NMR shifts of monolignol glucosides and intermediates in their syntheses

	IIIa	IIIb	IIIc	VIIIa	VIIIb	VIIIc	VIIa	VIIb	VIIc
α	191.38	191.49	191.77	143.54	143.90	144.26	127.95	128.27	128.31
β	–	–	–	116.70	117.12	118.00	128.69	128.86	130.03
γ	–	–	–	166.20	166.21	166.22	61.48	61.47	61.34
OMe	–	55.91	56.23	–	55.98	56.29	–	55.53	56.24
1	131.28	131.86	132.48	128.76	129.80	130.69	130.56	130.89	132.47
2	131.62	111.39	106.65	129.98	111.91	106.04	127.03	109.78	104.36
3	116.40	149.78	152.89	116.45	149.73	152.71	116.20	148.90	152.57
4	160.68	150.52	138.72	157.77	147.40	135.62	156.59	145.85	133.75
5	116.40	116.40	152.89	116.45	117.26	152.71	116.20	115.16	152.57
6	131.62	124.52	106.65	129.98	121.94	106.04	127.03	118.87	104.36
1′	96.27	97.51	99.98	96.59	98.09	100.32	100.26	99.91	102.46
2′	70.49	70.48	71.40	70.56	70.56	71.47	73.12	73.09	74.06
3′	71.79	71.74	70.77	71.84	71.76	72.04	76.91	76.89	77.08
4′	67.85	67.87	68.07	67.94	67.98	68.19	69.60	69.55	69.83
5′	70.95	70.95	71.96	70.83	70.81	70.71	76.49	76.73	76.43
6′	61.47	61.48	61.65	61.54	61.53	61.76	60.59	60.56	60.79
Ac Me	20.20	20.19	20.22	20.22	20.21	20.29	–	–	–
Ac Me	20.20	20.19	20.22	20.22	20.21	20.36	–	–	–
Ac Me	20.29	20.28	20.28	20.29	20.28	20.36	–	–	–
Ac Me	20.37	20.36	20.33	20.39	20.39	20.36	–	–	–
Ac C = O	168.96	168.81	168.87	168.96	168.83	168.96	–	–	–
Ac C = O	169.47	169.45	169.44	169.46	169.44	169.52	–	–	–
Ac C = O	169.18	169.16	169.18	169.19	169.18	169.26	–	–	–
Ac C = O	169.83	169.82	169.76	169.83	169.84	169.84	–	–	–
CH_2	–	–	–	59.81	59.81	59.93	–	–	–
CH_3	–	–	–	14.14	14.12	14.18	–	–	–

4-O-[Tetra-O-acetyl-β-D-gluco]-ferulic acid ethyl ester (VIIIb) and 4-O-[tetra-O-acetyl-β-D-gluco]-sinapic acid ethyl ester (VIIIc)

Using the procedure described for VIIIa, compounds VIIIb and Vlllc were prepared in yields of 94% and 93% respectively; mp. 134-137°C (lit. 139°C, Kratzl and Billek 1953), m/z (M + NH_4^+) = 570 (VIIIb), mp. 155–156°C, m/z (M + NH_4^+) = 600 (VIIIc).

p-Glucocoumaryl alcohol (VIIa)

Compound VIIIa (3.14g, 6mMol) was dissolved in dry toluene (200 ml, Aldrich Chemical Co.) and diisobutylaluminum hydride (DIBAL-H, 1.5M in toluene, 50ml, 75mMol, Aldrich Chemical Co,) was slowly added with stirring under nitrogen at 0-5°C (ice-water cooling) over one hour. After the addition of DIBAL-H was complete, stirring was continued for another hour. The reaction mixture was then carefully quenched by slow addition of ethanol (20ml) with stirring while cooling. The solvents were removed thoroughly under vacuum at 50°C. Water (100ml) was added to the residue, and the small amount of toluene separated on the water layer was removed under reduced pressure at 50°C. Then the aqueous suspension was warmed in boiling water bath and filtered. The extraction with hot water (100 ml) was repeated twice. The combined water solutions were concentrated under reduced pressure (60°C) to give crystals of compound VIIa. Recrystallization from hot water gave white needles. The crude glucoside in the mother liquor was purified by column chromatography employing silica gel (Aldrich, Davisil, 100–200 mesh) and acetone-ethylacetate-water (10/10/1) as an eluent. Total yield, 1.61g. 86%: mp. 177-179°C (lit. 180–182°C, Kratzl and Billek 1954). m/z(M+NH_4^+)=330.

Coniferin (VIIb), and syringin (VIIc)

Coniferin and syringin were prepared by the same procedure as described above for preparation of*p* -glucocoumaryl alcohol. VIIb: mp. 183–185°C (lit. 183–185°C, Kratzl and Billek 1953b), m/z (M+ NH_4^+) = 360. Yield; 69%; VIIc: mp. 191-192°C (lit. 190-191°C, Kratzl and Billek 1954), m/z (M + NH_4^+) = 390. Yield; 87%. Coniferin and syringin synthesized by the present method showed no melting point depression when mixed with coniferin and syringin, respectively, isolated from the cambium of spruce and lilac, respectively.

Results and Discussion

In the conventional method for preparation of monolignols glucosides, glucosides of cinnamic acids have been converted into corresponding acid chlorides. To obtain high yields in the reduction step with sodium trimethoxyborohydride (Fig. 1), it was recommended to purify the acid chlorides by recrystallization. However, the purification of the moisture sensitive acid chlorides and the final deacetylation step with sodium methoxide cause decreased yield. Radiolabeled monolignols glucosides are usually prepared on a small scale, and it is particularly important to reduce losses in this type of preparation. The new method described in this paper requires fewer steps and gives higher yields than previous methods.

For the preparation of monolignols glucosides labeled at side chain carbons, ^{14}C-malonic acid or ^{13}C-malonic acid have been often used as starting materials (Terashima *et al.* 1988, 1991). Condensation of compounds III with malonic acid can be accomplished by the same technique as that used with malonic acid monoethyl ester (Fig. 2). To increase the incorporation of isotope into the side chain, it is recommended that the molar ratio of labeled malonic acid to aldehyde be slightly lower than 1. After most of the labeled malonic acid has reacted, unlabeled malonic acid is then added to increase the yield of the condensation product. In addition, after the reaction mixture has been poured into water, dilute hydrochloric acid should be added to make the solution slightly acidic and thus precipitate the cinnamic acid glucoside tetraacetate completely. The esterification of the acid to give esters VIII (of methyl equivalent) can be achieved by refluxing a mixture of the acid, methyl iodide (2.5 equiv.) and potassium carbonate (1.5 equiv.) in dry acetone for 3 hours. After evaporation of solvent and excess methyl iodide, the residue is dissolved in chloroform. The chloroform solution is washed with water and dried with anhydrous magnesium sulfate. The residue obtained after evaporation of chloroform is recrystallized from methanol. The methyl ester can be converted corresponding monolignol glucosides in good yield by the new procedure employing DIBAL-H.

A particular advantage of the new procedure described here is that excess reduction or incomplete reduction do not occur to give byproducts with saturated side chains or with a carbonyl group at the terminal γ-side chain carbon. This was evident from the NMR spectra of the products. Complete proton and carbon chemical shift data for the products and intermediates are given in Tables 1 and 2.

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