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Tetrahedron Letters 47 (2006) 695–699
Tetrahedron Letters

Glycosylation of vanillin by amyloglucosidase in organic media

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Received 25 August 2005; revised 17 November 2005; accepted 23 November 2005
Available online 7 December 2005

Abstract—Glycosylation of vanillin using amyloglucosidase from a *Rhizopus* mold with D-glucose, D-galactose, D-mannose, maltose, sucrose and D-sorbitol in di-isopropyl ether yielded glycosides in the range 13–53%. NMR spectral data confirmed linking between the phenolic OH of vanillin and C1 and/or C6 of the carbohydrate moieties.

Vanillin **1** (4-hydroxy-3-methoxybenzaldehyde)[1] is used as an additive in food and beverages (60%), considerable amounts as flavour and fragrances (20–25%) and 5–10% as an intermediate for pharmaceuticals. It possesses a wide range of pharmacological activities such as antimicrobial,[2] anticarcinogenic,[3] antioxidant,[4] antifungal[5] and antimutagenic.[6] The solubility of vanillin in water varies from 3 g/l at 4.4 °C to 62.5 g/l at 80 °C.[7] Thus the solubility and bioavailability of **1** limits its pharmacological applications. Glycosylation is a useful tool to improve the water solubility and bioavailability[8,9] of vanillin.

The preparation of vanillin glycosides has been reported by cell suspension culture,[9] chemical[10] and plant cell tissue and organ culture methods.[11] However, preparations by enzymatic methods have not been previously reported. The present work describes an enzymatic method using amyloglucosidase from a *Rhizopus* mold for the preparation of glycosides with mono- and disaccharides in a non-polar solvent.

A typical synthesis involved reacting **1** (0.0005–0.0025 mol) and a mono- or disaccharide (**2**–**7**, 0.0005–0.0025 mol) at reflux with stirring in 100 ml of di-isopropyl ether in the presence of 10–80% (by weight of **2**–**7**) amyloglucosidase and 0.1–1.0 ml of 10 mM of pH 4.0–8.0 buffer for a period of 72 h. Refluxing di-isopropyl ether for 72 h did not produce any peroxides. Work-up involved distilling off the solvent and maintaining the reaction mixture at boiling water temperature for 5–10 min to denature the enzyme. The residue was repeatedly extracted with chloroform to remove unreacted **1** and the dried residue, consisting of vanillin glycoside and unreacted mono- or disaccharide, was subjected to HPLC analysis on an amino-propyl column (3.9 × 300 mm length), eluted with 80:20 (v/v) acetonitrile/water with a flow rate of 1 ml/min and monitoring with a RI detector. Conversion yields were determined from HPLC peak areas of the glycoside and free mono- or disaccharides and expressed with respect to mono- or disaccharide concentrations. Errors based on the HPLC measurements were of the order ±10%. Glycosides were isolated using a Sephadex G25 (100 cm × 1 cm) column, eluting with water and subjected to spectroscopic characterization.

Glycosylation of **1** was carried out with the following mono- and disaccharides: pentoses—D-ribose and D-arabinose; hexoses—D-glucose **2**, D-galactose **3** and D-mannose **4**; ketoses—D-fructose; and disaccharides—maltose **5**, lactose and sucrose **6**; carbohydrate alcohols—D-mannitol and D-sorbitol **7**. Amyloglucosidase exhibited maximum activity in non-polar solvents containing a certain minimum amount of water to stabilize its active conformation in a non-polar solvent compared to other enzymes.[12,13] Hence, glycosylation of **1** was always carried out in the presence of buffers of certain pH and concentration, worked out from a series of reactions conducted with different pH, buffer concentrations, substrate concentration, amyloglucosidase concentration and incubation period. Optimized reaction conditions for the preparation of vanillin glucoside at equimolar concentrations of **1** and **2** were found to be

Keywords: Amyloglucosidase; Disaccharides; Glycosylation; Monosaccharides; Organic media; Vanillin glycosides.
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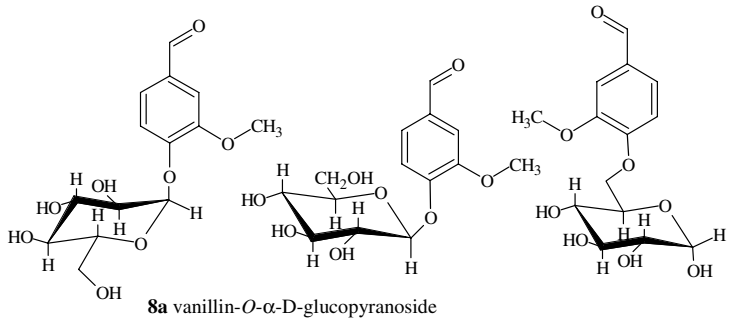
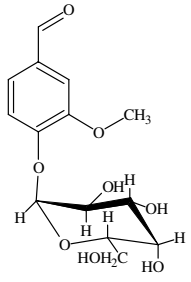
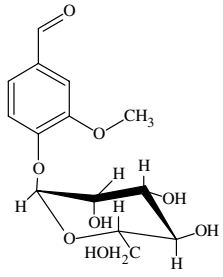
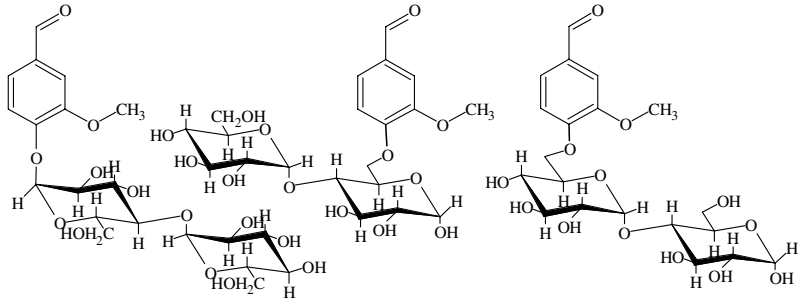
doi:10.1016/j.tetlet.2005.11.114

Table 1. Synthesis of vanillin glycosides with mono- and disaccharides (**2**–**7**)[a]

Glycosides	Glycosylation yield[b] (%)	Product (% proportion[c])
 **8a** vanillin-*O*-α-D-glucopyranoside **8b** vanillin-*O*-β-D-glucopyranoside **8c** vanillin-*O*-6-α-D-glucopyranoside	53	C1α (52), C1β (17) and C6α (31) mono-glucosides
 **9** vanillin-*O*-α-D-galactopyranoside	18	C1α galactoside
 **10** vanillin-*O*-α-D-mannopyranoside	13	C1α mannoside
 **11a** vanillin-*O*-α-maltopyranoside **11b** vanillin-*O*-6-α-maltopyranoside **11c** vanillin-*O*-6″-α-maltopyranoside	29	C1 (42), C6 (30) and C6″ (28) mono-maltosides