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Syntheses of Retinol Glycosides Using β-glucosidase in $SCCO_2$ Media

Rajachristu Einstein Charles · Thangavel Ponrasu · Kadimi Udaya Sankar · Soundar Divakar

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Abstract β-Glucosidase isolated from sweet almond catalyzed syntheses of water soluble retinol glycosides were carried out in $SCCO_2$ media with carbohydrates—D-glucose **2**, D-galactose **3**, D-mannose **4**, D-fructose **5**, and D-sorbitol **6**. Retinol glycosides yields were in the 9–34% range. Reaction with D-fructose **5** gave a highest yield of 34%. Excellent regioselectivity was observed with D-mannose **4** and D-sorbitol **6** which gave exclusively C1β-mannoside and C1-D-sorbitolide.

Keywords β-glucosidase · Regioselectivity · Retinol · Retinol glycoside · Supercritical carbon dioxide media ($SCCO_2$) · Enzymatic catalysis

Introduction

Components of vitamin A, retinol and retinoic acid, are fat soluble micronutrients essential for growth, vision, cell differentiation, and integrity of the immune system [1–3]. Its derivatives show therapeutic utility in several types of cancer and skin disorders [4]. Different metabolic forms of vitamin A show different activities—11-cis retinol is the major ligand for the opsins in vision. 9-cis retinoic acid is active in cell differentiation and embryogenesis and retinyl esters serve as transport and storage forms of vitamin A [4]. The need to synthesis and evaluate newer and more efficient glycosides of vitamins including vitamin A is strong due to several factors including unmet therapeutic needs, remarkable diversity of both chemical structures and biological activities of naturally occurring

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R. E. Charles · T. Ponrasu · S. Divakar (✉)
Fermentation Technology and Bioengineering, Central Food Technological Research Institute, Council of Scientific and Industrial Research, Mysore 570 020, India
e-mail: divakar643@gmail.com

K. U. Sankar
Food Engineering Department, Central Food Technological Research Institute, Council of Scientific and Industrial Research, Mysore 570 020, India

secondary metabolites, the utility of bioactive natural products as biochemical and molecular probes and advances in solving the demand for supply of complex natural products [5]. One such glycosidic derivative of retinol, *N*-(4-hydoxyphenyl)retinamide (4-HPR) has been shown to be effective in numerous types of tumor models and has been included in phase III clinical trials also [2, 6].

Need for various protection–deprotection steps along with safe handling of vitamin A due its air and light sensitive nature has rendered the synthetic methods very expensive. Vanderjagt et al. [7] have synthesized retinol glycosides through a transglycosylation reaction between glucocerebroside and retinol. Glycosylation of fat-soluble vitamins [8] into water-soluble form has been carried out successfully by reverse hydrolytic method [9, 10]. Enzymatic methods offer ideal reaction conditions for the synthesis of compounds like vitamin A which are unstable. Enzymatic methods involve milder reaction conditions, easy workup, good yields, and high selectivity. Our enzymatic reactions with vitamin A in shake flasks resulted in degradation of vitamin A. Hence, still more mild conditions are required for compounds like vitamin A. Supercritical fluid carbon dioxide media ($SCCO_2$), was employed. Besides maintaining the integrity of the compounds during reaction, it would also be cost effective to carry out such reactions in $SCCO_2$ media. Performing reactions in supercritical fluids (SCF) with carbon dioxide also have other advantages like energy reduction, ease of product recovery, lesser cost of downstream processing and reduction in side reactions [11–13]. Until today, reports on enzymatic glycosylation involving $SCCO_2$ are very scanty if not nil. The present work describes an enzymatic method for the preparation of retinol glycosides using β-glucosidase from sweet almond in $SCCO_2$ media.

Materials and Methods

Enzyme and Chemicals

Retinol was purchased from Sigma, St. Louis, MO, USA and β-glucosidase was isolated from sweet almond [14]. About 1 kg of finely powdered defatted sweet almond powder was dispersed in a solution of 50 g of $ZnSO_4$. $7H_2O$ in 4 L of water and left standing at 0 °C for 4 to 5 h. The cold solution was then filtered through cloth and well pressed on the filter. To the filtrate was cautiously added a solution of 1.4 g tannin (0.28%) in 500 mL water. A precipitate consisting mostly of impurities was removed by centrifugation and discarded. The bulk of the enzyme was then precipitated slowly by adding 500 mL of 3% tannin solution (15 g/500 mL) in water. The precipitate was removed by centrifugation, freed from tannin by repeatedly dispersing it in acetone and centrifuging it to get crude powder. The crude powder was dialyzed using 3.5 kDa membrane and finally lyophilized to get a dry powder. β-glucosidase activity was found to be 3.12 AU-mmol/mg/enzyme preparation/min [15] and specific activity 4.058 AU-mmol/min/mg protein. D-Galactose and D-fructose procured from HiMedia, India; D-glucose from SD Fine Chemicals (Ind.); D-sorbitol from Loba Chemie, India were employed. Dimethylformamide from Qualigens Fine Chemicals (Ind.), India and HPLC grade acetonitrile from Sisco Research Laboratories, India were employed after distilling once.

Gel Electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a discontinuous buffer system to check the purity of β-glucosidase employed in the present work

[16]. β-Glucosidase showed a single band of molecular mass 64.6 kDa (Fig. 1) which is in good correspondence with the one active component of molecular mass 66.5 kDa [17]. Although, the partially purified enzyme was found to be free from contaminants, the enzyme also exhibited an α-glucosidase activity of 359.7 AU-(μmol/min/mg enzyme preparation).

Glycosylation Reaction in $SCCO_2$ Media

Syntheses of retinol glycosides were carried out under $SCCO_2$ pressure of 120 bar at 50 °C. A reaction vessel of 100 ml capacity controlled thermostatically fitted with a magnetic stirrer and a recirculating fluid pressure differential loop with a Rheodyne valve capable of delivering 0.5 ml sample, was employed. Retinol **1** (0.5 mmol), carbohydrate **2–6** (1 mmol), 40% (*w*/*w* of carbohydrate) β-glucosidase, 0.12 mM (1.2 ml), pH 6 phosphate buffer and 24-h incubation period in 10 ml of DMF under 120 bar $SCCO_2$ at 50 °C was employed for the reactions. A concentration of 40% (*w*/*w* carbohydrate) of β-glucosidase corresponded to 224.6 AU of the enzyme. After the reaction, carbon dioxide was released slowly and the enzyme denatured at 100 °C by holding in boiling water bath for 5–10 min. The reaction

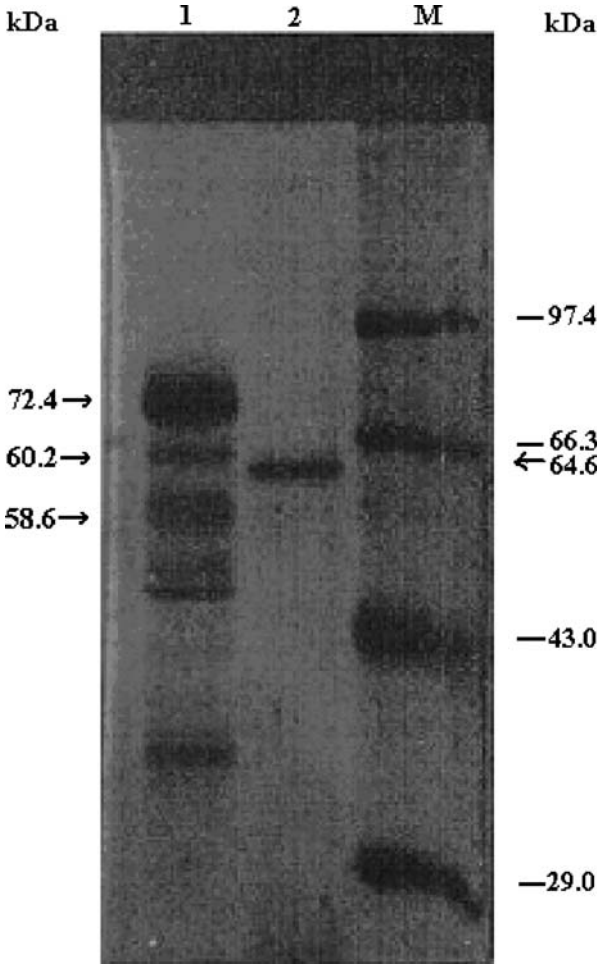


Fig. 1 SDS-PAGE lane *1* for amyloglucosidase from *Rhizopus* sp. (from Sigma), lane *2* for β-glucosidase isolated from sweet almond, lane *M* for M_r standard proteins: phosphorylase (97.4 kDa), BSA (66.3 kDa), ovalbumin (43.0 kDa) and carbonic anhydrase (29.0 kDa)

mixture was extracted with hexane to remove unreacted retinol **1**. The aqueous reaction mixture was evaporated in dark to get a mixture of the glycoside and unreacted carbohydrate. Retinol being light and air sensitive, work-up and isolation was also carried out in dark.

Glycosides were analyzed by HPLC in dark using an aminopropyl column (250× 4.6 mm) and acetonitrile–water in 70:30 ratio (*v*/*v*) as the mobile phase at a flow rate of 1 ml/min using refractive index detector (Fig. 2). Conversion yields were determined from HPLC peak areas of the glycoside and free carbohydrate with respect to the free carbohydrate employed. Percentage of the glycoside peak area as a ratio of the total area of the carbohydrate and the glycoside signals was expressed as the conversion yield. Error in HPLC measurements will be ±5%. Glycosides formed were separated through size exclusion chromatography under dark using Sephadex G15 column (100×1 cm) eluting with water. Even though the glycosides were separated from unreacted carbohydrate, individual glycosides could not be separated from their reaction mixtures due to similar molecular weights of the glycosides formed. HPLC retention times for retinol and its glycosides are: retinol—3.5 min, D-glucose—7.5 min, 18-*O*-(D-glucopyranosyl)retinol—8.9 min, D-galactose—7.0 min, 18-*O*-(D-galactopranosyl) retinol—7.6 min, D-mannose—6.7 min, 18-*O*-(β-D-mannopyranosyl)retinol—7.7 min, D-fructose—6.8 min, 18-*O*-(D-fructofuranosyl) retinol—7.9 min, D-sorbitol—6.7 min, and 18-*O*-(1-D-sorbital) retinol—7.7 min.

Product Characterization

Isolated glycosides were characterized by measuring melting point and optical rotation wherever it was necessary and by recording UV, IR, MS, and 2D heteronuclear single quantum coherence transfer (2D-HSQCT) spectra. Mass spectra also confirmed the formation of monoglycosides of retinol. The nature and proportion of products were confirmed by 2D HSQCT NMR. Glycosides of retinol exhibited surfactant property and hence at the concentrations employed for 2D HSQCT, which were above the critical micellor concentrations, the proton NMR signals were unusually broad such that, in spite of recording the spectra at 35 °C, the individual coupling constant values could not be determined precisely. Only resolvable signals are shown. Some of the assignments are interchangeable.

18-*O*-(D-Glucopyranosyl)retinol **7a,b**

Solid; isolated yield—0.035 g (15.6%) UV (H_2O, λ_{max}): 202 nm (σ→σ*, ε_{202}—1114 M^{-1}), 227.5 nm (σ→π*, $\varepsilon_{227.5}$—892 M^{-1}), 260.5 nm (π→π*, $\varepsilon_{260.5}$—629 M^{-1}), 286.5 nm

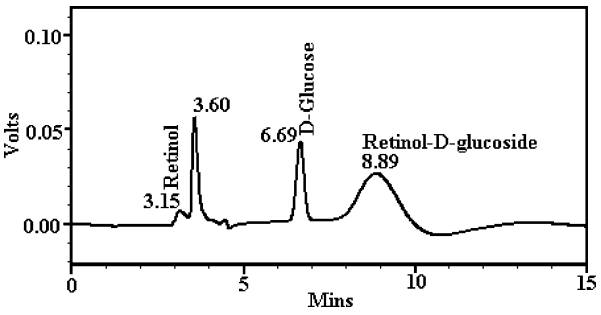


Fig. 2 HPLC chromatogram for the reaction mixture of D-glucose, retinol and 18-*O*-(D-Glucopyranosyl)retinol. HPLC conditions: aminopropyl column (10 μm, 300×3.9 mm), solvent-CH_3CN: H_2O (70:30 *v*/*v*), flow rate—1 mL/min, RI detector. Retention times: retinol—3.15 min, D-glucose—6.69 min and 18-*O*-(D-Glucopyranosyl) retinol—8.89 min

($n\rightarrow\pi^*$, $\varepsilon_{286.5}$—612 M^{-1}); IR (stretching frequency, cm^{-1}): 1,026 (C-O-C alkyl alkyl symmetrical), 1,256 (C-O-C alkyl alkyl asymmetrical), 3,390 (OH), 1,456 (aromatic C=C), 2,939 (CH); MS (*m/z*) 449 $[M+1]^+$; 2D-HSQCT (DMSO-d_6) **C1α-glucoside 7a**: ^{1}H NMR δ_{ppm} (500.13 MHz) **Glu**: 4.04 (H-1α, d, J=2.9 Hz), 3.45 (H-3α), 2.93 (H-4α), 3.48 (H6-α), **Retinol**: 6.18 (H-2), 6.12 (H-5), 6.70 (H-6), 6.09 (H-9), 6.31 (H-10), 6.20 (H-11), 1.87 (H-12), 1.71 (H-14), 1.09 (H-15, 16), 1.44 (H-17), 4.51 (H-18), 1.92 (H-19), 2.02 (H-20), ^{13}C NMR δ_{ppm} (125 MHz) **Glu**: 97.1 (C-1α), 75.5 (C3-α), 71.0 (C-4α), 63.5 (C6-α) **Retinol**: 129.3 (C-3), 34.5 (C-4), 131.5 (C-6), 131.7 (C-9), 129.6 (C-11), 33.4 (C-12), 40.1 (C-13), 19.8 (C-14), 29.2 (C-15, 16), 19.7 (C-17), 64 (C-18), 12.9 (C-19), 12.5 (C-20), **C1β-glucoside 7b:** ^{1}H NMR δ_{ppm} **Glu**: 4.89 (H-1β, d, J=7.3 Hz), 2.74 (H-2β), 3.15 (H3-β), 3.45 (H3-β), ^{13}C NMR δ_{ppm} **Glu**: 103.4 (C-1β), 78.3 (C-2β), 77.0 (C3-β), 76.5 (C-5β).

18-*O*-(D-Galactopyranosyl)retinol **8a,b**

Solid; isolated yield—0.051 g (22.8%) UV (H_2O, λ_{max}): 196 nm ($\sigma\rightarrow\sigma^*$, ε_{196}—990 M^{-1}), 214 nm ($\sigma\rightarrow\pi^*$, ε_{214}—749 M^{-1}), 260 nm ($\pi\rightarrow\pi^*$, ε_{260}—661 M^{-1}), 280 nm ($n\rightarrow\pi^*$, ε_{280}—649 M^{-1}); IR (stretching frequency, cm^{-1}): 1,068 (C-O-C alkyl alkyl symmetrical), 1,298 (C-O-C alkyl alkyl asymmetrical), 3,384 (OH), 1,428 (aromatic C=C), 2,937 (CH); MS (*m/z*) 449 $[M+1]^+$; 2D-HSQCT (DMSO-d_6) **C1α-galactoside 8a:** ^{1}H NMR δ_{ppm} (500.13 MHz) **Gal**: 4.30 (H-1α, d, J=3.4 Hz), 3.61 (H-3α), 3.74 (H-4α), 3.55 (H6-α), **Retinol**: 5.71 (H-5), 6.65 (H-10), 6.15 (H-11), 1.47 (H-12), 1.49 (H-13), 4.31 (H-18), ^{13}C NMR δ_{ppm} (125 MHz) **Gal**: 95.7 (C-1α), 69.1 (C-3α), 71.3 (C-4α), 71.1 (C-5α), 61.6 (C-6α), **Retinol**: 124.5 (C-2), 129.2 (C-3), 131.6 (C-6), 138.5 (C-10), 129.3 (C-11), 29.5 (C-15, 16), 19.7 (C-17), 63 (C-18), 12.5 (C-20), **C1β-galactoside 8b:** ^{1}H NMR δ_{ppm} **Gal**: 5.01 (H-1β, d, J=7.9 Hz), 3.17 (H-3β), 3.34 (H-5β), 3.35 (H6-β), ^{13}C NMR δ_{ppm} **Gal**: 102.2 (C-1β), 77.1 (C-2β), 73.4 (C-3β), 72.1 (C-4β), 75.1 (C-5β), 62.9 (C-6β).

18-*O*-(β-D-Mannopyranosyl)retinol **9**

Solid; isolated yield—0.032 g (14.3%) mp. 110 °C, UV (H_2O, λ_{max}): 199.5 nm ($\sigma\rightarrow\sigma^*$, $\varepsilon_{199.5}$—810 M^{-1}), 218 nm ($\sigma\rightarrow\pi^*$, ε_{218}—742 M^{-1}), 237.5 nm ($\sigma\rightarrow\pi^*$, $\varepsilon_{237.5}$—725 M^{-1}), 261.5 nm ($\pi\rightarrow\pi^*$, $\varepsilon_{261.5}$—576 M^{-1}), 281 nm ($n\rightarrow\pi^*$, ε_{281}—574 M^{-1}); IR (stretching frequency, cm^{-1}): 1,068 (C-O-C alkyl alkyl symmetrical), 1,243 (C-O-C alkyl alkyl asymmetrical), 3,333 (OH), 1,380 (aromatic C=C), 2,933 (CH); optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25 °C=-14.3; MS (*m/z*) 449 $[M+1]^+$; 2D-HSQCT (DMSO-d_6) **C1β-mannoside**: ^{1}H NMR δ_{ppm} (500.13 MHz) **Man**: 5.01 (H-1β, d, J=3.3 Hz), 3.70 (H3-β), 3.64 (H-4β), 78.5 (H5-β), 3.55 (H6-β) **Retinol**: 1.28 (H-12), 1.11 (H-13), 1.01 (H-15, 16), 1.15 (H-17), 4.30 (H-18), ^{13}C NMR δ_{ppm} (125 MHz) **Man**: 101.8 (C-1β), 71.3 (C-2β), 78.1 (C-3β), 70.1 (C-4β), 79.2 (C-5β), 67.5 (C-6β), **Retinol**: 142.6 (C-10), 29.2 (C-15), 29.2 (C-16), 19.7 (C-17), 63.6 (C-18), 12.9 (C-19), 12.5 (C-20).

18-*O*-(D-Fructofuranosyl)retinol **10a,b**

Solid; isolated yield—0.055 g (24.6%) UV (H_2O, λ_{max}): 203 nm ($\sigma\rightarrow\sigma^*$, ε_{203}—1223 M^{-1}), 220.5 nm ($\sigma\rightarrow\pi^*$, $\varepsilon_{220.5}$—1074 M^{-1}), 260.5 nm ($\pi\rightarrow\pi^*$, $\varepsilon_{260.5}$—515 M^{-1}), 281 nm ($n\rightarrow\pi^*$, ε_{281}—482 M^{-1}); IR (stretching frequency, cm^{-1}): 1,059 (C-O-C alkyl alkyl symmetrical), 1,237 (C-O-C alkyl alkyl asymmetrical), 3,350 (OH), 1,411 (aromatic C=C), 2,937 (CH); MS (*m/z*) 449 $[M+1]^+$; 2D-HSQCT (DMSO-d_6) **C1-*O*-fructoside 10a:** ^{1}H NMR δ_{ppm} (500.13 MHz) **Fru**: 3.75 (H-1), 77.5 (H-2), 72.5 (H-3), 3.59 (H-5), **Retinol**:

1.91 (H-12), 1.23 (H-13), 1.05 (H-15, 16), 4.45 (H-18), ^{13}C NMR δ_{ppm} (125 MHz) **Fru**: 62.5 (C-1), 77.5 (C-2), 72.5 (C-3), 73.5 (C-4), 71.5 (C-5), **Retinol**: 35.9 (C-4), 30.9 (C-12), 63.6 (C-18), **C6-*O*-fructoside 10b:** ^{1}H NMR δ_{ppm} **Fru**: 3.32 (H-4), 3.75 (H-6), ^{13}C NMR δ_{ppm} **Fru**: 69.5 (C-4), 64.5 (C-6).

18-*O*-(1-D-Sorbitol)retinol **11**

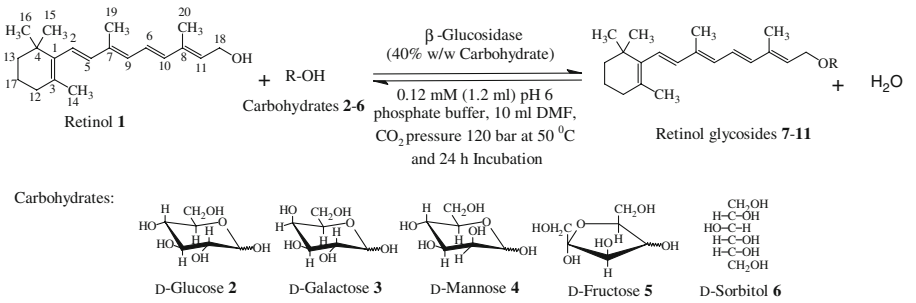
Solid; isolated yield—0.011 g (4.5%) mp. 115 °C, UV (H_2O, λ_{max}): 205 nm ($\sigma\rightarrow\sigma^*$, ε_{205}—940 M^{-1}), 219.5 nm ($\sigma\rightarrow\pi^*$, $\varepsilon_{219.5}$—1,645 M^{-1}), 261 nm ($\pi\rightarrow\pi^*$, ε_{261}—667 M^{-1}), 288 nm ($n\rightarrow\pi^*$, ε_{288}—753 M^{-1}); IR (stretching frequency, cm^{-1}): 1,080 (C-O-C aryl alkyl symmetrical), 1,220 (C-O-C aryl alkyl asymmetrical), 3,367 (OH), 1,413 (aromatic C=C), 2,935 (CH); optical rotation (*c* 0.5, H_2O): $[\alpha]_D$ at 25 °C=+6.4; MS (*m/z*) 451 $[M+1]^+$; 2D-HSQCT (DMSO-d_6) **C1-*O*-sorbitol**: ^{1}H NMR δ_{ppm} (500.13 MHz) **Sor**: 3.42 (H-1), 3.70 (H-3), 3.41 (H-4), 3.69 (H-5), 3.61 (H-6), **Retinol**: 6.15 (H-10), 5.72 (H-11), 1.28 (H-12), 1.18 (H-13), 1.05 (H-15, 16), 1.25 (H-17), ^{13}C NMR δ_{ppm} (125 MHz) **Sor**: 62.2 (C-1), 73.3 (C-4), 73.1 (C-5), **Retinol**: 35.9 (C-4), 137.5 (C-10), 130.2 (C-11), 31.3 (C-12), 40.4 (C-13), 29.2 (C-15), 29.5 (C-16), 19.7 (C-17), 60.5 (C-18), 12.5 (C-20).

Results and Discussion

18-*O*-(D-Glucopyranosyl)retinol

Glycosylation did not take place without the enzyme in $SCCO_2$, under the reaction conditions of temperature and low solvent content employed. Conducting the reaction in shake flasks, under such reaction conditions gave degraded products of vitamin A. Hence, carrying out reactions in $SCCO_2$ was found to be extremely advantageous as the conditions employed were milder and the yield and selectivity were extremely good.

Glycosylation reaction was carried out between retinol **1** and carbohydrates—aldohexoses—D-glucose **2**, D-galactose **3**, and D-mannose **4**; ketohexose—D-fructose **5**; and carbohydrate alcohol—D-sorbitol **6** (Scheme 1). The reaction conditions employed were worked out after preliminary investigations. The reaction took place in a facile manner and the yields obtained were in the 9% to 34% range. Highest yield of 34% was obtained with D-fructose.

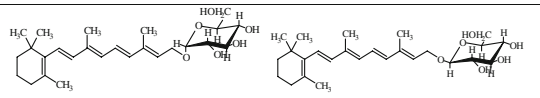
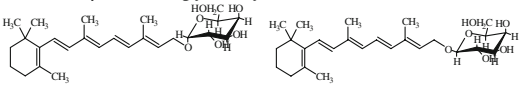
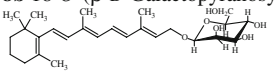
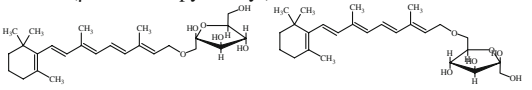
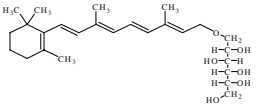


Scheme 1 Syntheses of retinol glycosides using β-glucosidase

Spectral Characterization

No oxidative or polymerized products of retinol were detected from the NMR spectra and Mass spectra. Ultraviolet-visible spectra of retinol glycosides, showed shifts in σ→σ* band in the 196–205 nm (192 nm for free retinol) range, σ→π* band in the 214–237.5 nm (236 nm for free retinol) range, π→π* band in the 260–261.5 nm (266.5 nm for free retinol) range and n→π* band in the 280–286.5 nm (286.5 nm for free retinol) range. IR glycosidic C-O-C symmetrical stretching frequencies were observed in the 1026–1080 cm^{-1} range and glycosidic C-O-C asymmetrical stretching frequencies in the 1,220–1,298 cm^{-1} range indicating that retinol had undergone glycosylation. From 2D HSQCT spectra of the retinol glycosides, the following glycoside formation were confirmed from their respective chemical shift values: from D-glucose **2** C1α glucoside **7a** to C1α at 97.1 ppm and H-1α at 4.04 ppm and C1β glucoside **7b** to C1β at 103.4 ppm and H-1β at 4.89 ppm; from D-galactose **3** C1α galactoside **8a** to C1α at 95.7 ppm and H-1α at 4.30 ppm and C1β galactoside **8b** to C1β at 102.2 ppm and H-1β at 5.01 ppm; from D-mannose **4** C1β mannoside **9** to C1β at 101.8 ppm and H-1β at 5.01 ppm; from D-fructose **5**

Table 1 β-Glucosidase catalyzed syntheses of retinol glycosides[a].

Glycosides	Product (% proportion)[a]	Yields (%)[b]
 **7a** 18-*O*-(α-D-Glucopyranosyl)retinol **7b** 18-*O*-(β-D-Glucopyranosyl)retinol	C1α (38) and C1β (62) glucoside	21
 **8a** 18-*O*-(α-D-Galactopyranosyl)retinol **8b** 18-*O*-(β-D-Galactopyranosyl)retinol	C1α (26) and C1β (74) galactoside	28
 **9** 18-*O*-(β-D-Mannopyranosyl)retinol	C1β mannoside	18
 **10a** 18-*O*-(1-D-Fructofuranosyl)retinol **10b** 18-*O*-(6-D-Fructofuranosyl)retinol	C1-*O*- (35) and C6-*O*- (65) derivatized	34
 **11** 18-*O*-(1-D-Sorbitol)retinol	C1-*O*-derivatized	9

[a] Product proportions were calculated from the area of respective proton signals

[b] Conversion yields were from HPLC with respect to free carbohydrate **2**–**6**. Error in yield measurements is ±5%

C1-*O*-derivatized **10a** to C1 at 66.5 ppm and H-1 at 3.62 ppm and C6-*O*-derivatized **10b** to C6 at 71.5 ppm and H-6 at 3.52 ppm; from D-sorbitol **6** C1-*O*-derivatized **11** to C1 at 62.2 ppm and H-1 at 3.42 ppm. Mass spectra also confirmed formation of the above mentioned glycosides. Thus two-dimensional HSQCT data clearly indicated that the glycosylation occurred at the 18-CH_2OH of the olefinic moiety of retinol and C1 position of D-glucose **2**, D-galactose **3**, D-mannose **4**, and the CH_2OH groups of D-fructose **5** and D-sorbitol **6**.

Retinol Glycosides

Other retinol glycosides synthesized under the optimized conditions in $SCCO_2$ media showed moderate yields (Table 1). Glycosylation gave rise to 38% of α-D-glucoside and 62% of β-D-glucoside compared to the 40:60 α–β anomeric composition of D-glucose **2** and 42% α-D-galactoside along with 58% β-D-galactoside compared to the 92:8 α–β anomeric composition of D-galactose **3**. In the oxo-carbenium ion mechanism for the glycosylation [18], a planar carbenium ion center formed with D-glucose **2** and D-galactose **3** is available for attack by the nucleophilic retinol olefinic OH from both the above and below the plane giving rise to a mixture of α/β anomeric products. Unlike D-glucose **2**, D-galactose **3** yielded more of the β product. β-Glucosidase catalysis gave exclusively C1β mannoside and C1-*O*-derivatized D-sorbitol, indicating its capability to exhibit excellent regioselectivity (Table 1). However, D-fructose gave reaction products from both the CH_2 OH groups. Presence of α-glucosidase in the enzyme could be responsible for formation of the α-glycosides in case of D-glucose and D-galactose.

β-Glucosidase did not catalyze the reaction with D-ribose, D-arabinose, maltose, sucrose, lactose, and D-mannitol. This could be stronger due to binding of retinol **1** to the active site of β-glucosidase than the above mentioned carbohydrate molecules, thereby preventing the facile transfer of these carbohydrate molecules to the nucleophilic olefinic OH of retinol **1**. Our work on the preparation of several glycosides with different aglycons showed that diverse reactivity of the carbohydrate molecules were with respect to the aglycons employed [19, 20]. With different aglycons, different reactivities were observed even under conventional reaction conditions. In most of the reactions carried out in our laboratory glycosylation did not take place with D-ribose, D-arabinose, lactose, and sucrose however, with very few exceptions. This type of selectivity is not due to the $SCCO_2$ conditions employed.

This could be the first report for the enzyme-mediated glycosylation of retinol **1** in $SCCO_2$ media. A one step synthesis involved milder reaction conditions, eliminating the need of protective and de-protective strategies to convert light and air sensitive, fat soluble retinol **1** into more stable and water soluble glycosyl derivative, to be useful as a pharmacologically and therapeutically active water soluble component of vitamin A. Such derivatization of vitamin A was possible due to the potentiality of β-glucosidase to act in $SCCO_2$ media.

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