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Research Article

Enzyme Catalyzed Synthesis of Serotonyl and Epinephryl Glycosides Using Amyloglucosidase from *Rhizopus* Mold

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ABSTRACT
Glycosylation of phenolic hydroxyl groups of serotonin and epinephrine, using an amyloglucosidase from *Rhizopus* mold was carried out with D-glucose, D-galactose**,** D-mannose and D-ribose in di-isopropyl ether solvent to yield 13-29% of glycosides in 48h. NMR spectroscopic data indicated that the reaction occurred between the phenolic -OH group of serotonin and C1α /β and / or 6-O-groups of D-glucose, D-galactose**,** D-mannose and D-ribose. In case of epinephrine, 4 –OH and 3 –OH groups reacted with C1 β anomer of D-glucose and C-1 α/β anomers of D-mannose.

***Key Words**:* Amyloglucosidase, glycosylation, Epinephrine, Serotonin, 6-O arylation, Selectivity.

INTRODUCTION
Currently, much attention has been focused on the pharmacological importance of serotonin (5-hydroxytryptamine) and epinephrine (adrenaline). Serotonin is primarily found in the gastrointestinal tract and central nervous system of humans as well as animals. In addition to animals, it is also found in insects, fungi and plants (Kang *et al.,* 2009). Serotonin is an important neurotransmitter in the central and peripheral nervous systems. It has been reported to modulate the regulation of a variety of major physiological functions including affective behavior, memory and thermo-regulation via the interaction at serotonin receptor subtypes (Hoyer and Martin 1997). Basically 80 percent of the human body's total serotonin is located in the enterochromaffin cells in the gut, where it is used to regulate intestinal movements (Berger *et al.,* 2009) and eventually finds its way out of tissues into the blood. Serotonin present in the blood then stimulates cellular growth to repair liver damage (Matondo *et al.,* 2009). In blood, serotonin is collected from plasma, by platelets which store it and activate it whenever platelets bind todamaged tissue to stop bleeding and aid in healing (Marieb *et al.,* 2009). Serotonin, which is also chemically unstable, has an important role to play in the mechanistic action of anti-psychotic agents, a topic of intense research, which promises better treatments for Schizophrenia in the forthcoming years (Jones and Blackburn 2002; Katrien *et al.,* 1997).

Epinephrine is a hormone and also a neurotransmitter (Berecek and Brody 1982). As a hormone, it acts on nearly all body tissues. Epinephrine's various functions results from its binding to a variety of adrenergic receptors triggering a number of metabolic changes. Binding to α-adrenergic receptors inhibits insulin secretion, stimulates glycogenolysis in the liver and muscle, and stimulates glycolysis in muscle (Sircar 2007). β-Adrenergic receptor binding triggers glucagons secretion in the pancreas, increased adreno corticotropic hormone secretion by the pituitary gland, and increased lipolysis by adipose tissue. All these effects lead to increased blood glucose and fatty acids, providing substrates for energy production within cells throughout the system (Sircar 2007). Due to its vasoconstrictive effects, epinephrine is the drug of choice for treating anaphylaxis and also useful in

treating sepsis. It is also used as a bronchodilator for asthma as its specific β-agonists are unavailable or ineffective.

Both serotonin and epinephrine can be made more water-soluble (Solubility of serotonin in water 2g/100ml at 27^{o}c that of epinephrine 0.01g/100ml at 18^{o}c) and stable by derivatising. Serotonin and epinephrine supplements especially derivatised ones could be better substituents for external administration in diet which is the principal objective of the present work.

Glycosylation is one way to synthesise bio-active derivatives of serotonin and epinephrine and it renders water solubility and stability to these molecules. Chemical methods of glycosylation are tedious as they involve several protective and deprotective steps, which result in cost intensive processes. Preparation of serotonin and epinephrine glycosides using enzyme has not been reported so far. Earlier works on enzymatic glycosylation in our laboratories have shown that several glycosides with diverse aglycons and carbohydrates could be prepared in good yield and

reasonable selectivity (Vijayakumar and Divakar 2005; Vijayakumar *et al.,* 2005; Sivakumar and Divakar 2009a; Sivakumar *et al.,* 2009b) through catalysis by enzymes in non-polar solvents. Hence enzymatic method is resorted to in the present work where serotonin and epinephrine glycosides are synthesized using amyloglucosidase from *Rhizopus* mold in di-isopropyl ether solvent media (Figure 1).

MATERIALS AND METHODS

Chemicals

Serotonin, epinephrine and amyloglucosidase from *Rhizopus* mold were purchased from Sigma-Aldrich Co., St. Louis, MO, and USA. Amyloglucosidase activity (Summer and Sisler 1944) was found to be 4.4-µmol mg protein^{-1} min^{-1}. Bio-Gel P-2 was purchased from Bio-Rad Laboratories, Inc. USA. All the carbohydrates and solvents were from Sisco Research Laboratories (P) Ltd., India. Solvents were distilled once before use.

Glycosylation Method

Equimolar quantity of serotonin **1** / epinephrine **2,** carbohydrates **3-6** (1mmole) were stirred under reflux in 100 ml di-isopropyl ether in presence of 40% w/w of amyloglucosidase to carbohydrate and 2 ml of 0.01M ph 6.0 phosphate buffer for 48 h at 68^{o}c. During work up, solvent was distilled off and the enzyme was denatured. The glycoside, unreacted carbohydrate and serotonin / epinephrine were taken in water and evaporated to obtain a residue. Isolated products were subjected to HPLC on an amino-propyl column 5µ (250 mm X 4.6 mm) with acetonitrile / water at 70:30 (v/v) as mobile phase under isocratic conditions at a flow rate of 1ml min^{-1} using refractive index as detector. Conversion yields were determined from HPLC peak areas of glycosides and unreacted carbohydrates. Error measurements in HPLC yields were $\pm$ 5-10 %. The retention times of serotonin, epinephrine and their respective glycosides are : D-glucose -5.8 min, D-galactose -6.7 min, D-mannose -6.0 min, D-ribose – 5.8 min, serotonin – 4.1 min, epinephrine – 5.1 min, serotonyl-D-glucoside -6.15 min, serotonyl-D-galactoside – 9.6 min, serotonyl-D-mannoside – 8.1 min, serotonyl-D-riboside – 6.5 min, epinephryl-D-glucoside – 7.3 min and epinephryl-D-mannoside – 7.7 min. Subsequently, the mixture was subjected to column chromatography to separate the glycosides. Bio-Gel P-2 Gel Fine 45-90µm packed in a 100 x 1cm length column was used for column chromatography with water as the eluent. Although unreacted aglycons and carbohydrate molecules could be separated by column chromatography, separation of individual components could not be achieved satisfactorily. The isolated products were subjected to UV, IR, 2D-NMR (HSQCT) that provided substantial information on the nature and proportions of the glycosides formed.

Characterization of products

Two-dimensional Heteronuclear Single Quantum Coherence Transfer Spectra (2DHSQCT) were recorded using a Brüker AQS 500 mhz NMR, (Fallanden, Switzerland) spectrometer operating at 500.18 mhz for ^{1}H and 125.78 mhz for ^{13}C at 20°C. Proton and carbon 90° pulse widths were 12.25 and 10.5 µs, respectively. Chemical shifts values were expressed in ppm relative to tetramethylsilane (TMS) as an internal standard. About 20 mg of the sample dissolved in DMSO-d_6 was used for recording the spectra. About 50-200 scans were accumulated with a recycle period of 2-3 seconds. A region from 0-10 ppm and 0-200 ppm were scanned for all the samples for ^{1}H and ^{13}C NMR, respectively. Mass spectra were obtained using a Q-TOF Waters Ultima instrument (Q-TQF GAA 082, Waters corporation, Manchester, UK) fitted with an electron spray ionization (ESI) source.

NMR spectra were recorded for the respective reaction mixtures to evaluate the proportion of individual components. In the NMR data only resolvable signals are shown. Some assignments are interchangeable as these glycosides act as surfactants, which tend to aggregate in solution giving broad signals. Hence in the proton part of 2DHSQCT coupling constant values including those of few anomeric protons could not be resolved satisfactorily. **Serotonyl 5-*O*-glucosides 7a-d**: Solid; Isolated yield- 30.8 mg (9.0%); UV(H_2O, λ_{max}) : 215nm ($\sigma\rightarrow\sigma^*$,$\epsilon_{215.0}$ -8730 M^{-1}), 275nm ($n\rightarrow\pi^*$,$\epsilon_{275.0}$ -2913M^{-1}), 370 ($n\rightarrow\pi^*$,$\epsilon_{370.0}$-1252 M^{-1}); IR (stretching frequency, cm^{-1}) 1080(C-O-C glycosidic aryl alkyl symmetrical), 1200(C-O-C glycosidic aryl alkyl asymmetric), 1415(aromaticc=C), 3427(OH); MS (*m/z*)-339 [M+1]$^{+}$; 2D HSQCT (DMSO-d_6): **C1-α-D-glucoside (7a) :** ^{1}H-NMR δ_{ppm}: (500.13 mhz): **Serot**: 6.93(H-4), 3.35(H-9), 2.86(H-10), **Glu:** 3.84(H-3α), 3.65(H-6α), 3.10(H-4α), **Serot**: 150.5(C-5), 128.4(C-4), 123.8(C-3), 51.3(C-10), 23.3(C-9); **Glu**: 95.9(C-1), 82.5 (C-3), 78.5(C-5), 72.1(C-4), 68.9(C-4), 62.7(C-6); **C1-β-D-glucoside (7b)** : ^{1}H-NMR δ_{ppm}: (500.13mhz): **Serot**: 7.25(H-3), 7.13(H-4), 6.83(H-6), 7.02(H-8), 3.20(H-9), 2.92(H-10); **Glu:** 3.93(H-3), 3.84(H-5), 3.82(H-4), 3.70(H-6); ^{13}C-NMR δ_{ppm} : **Serot**: 150.5(C-5), 128.4(C-4), 123.8(C-3), 52.8(C-10), 23.3(C-9); **Glu**:101.8(C-1), 73.4(C-5), 75.7(C-3), 73.2(C-4), 69.2(C-2), 64.0(C-6); **6-O-aryl-α-D-glucoside (7c):** ^{1}H-NMR δ_{ppm}: **Serot:** 3.54(H-6), 3.10(H-9); **Glu** 3.45(H-2), 3.62(H-4), 3.58(H-6);

^{13}C-NMR δ_{ppm} **Serot**: 52.6(C-10); **Glu**: 92.4(C-1), 62.7(C-6); **6-O-aryl-β-D-glucoside (7d)**: ^{1}H-NMR δ_{ppm}: **Serot:** 7.24(H-4), 3.14(H-9); **Glu:** 3.72(H-4), 3.86(H-6); ^{13}C- NMR δ_{ppm} : **Serot:** 52.2(C-10); **Glu:** 99.6(C-1), 62.6(C-6).

Serotonyl 5-*O*-galactosides 8a-c: Solid; Isolated yield- 5.3mg(7.5%); UV(H_2O,λ_{max}): 213nm ($\sigma\rightarrow \pi^*$,$\epsilon_{213.0}$ - 7878 M^{-1}), 278nm ($n\rightarrow\pi^*$,$\epsilon_{278.0}$ -4707 M^{-1}); IR(stretching frequency, cm^{-1}), 1016(C-O-C glycosidic aryl alkyl asymmetrical),1204(C-O-C glycosidic aryl alkyl symmetric), 1459 (aromatic C=C), 3307(OH); MS (*m/z*) 339 $[M+1]^+$; 2D HSQCT (DMSO-d_6): **C1-β-D-galactoside (8a)** : ^{1}H-NMR δ_{ppm}(500.13 mhz): **Serot**: 7.12(H-3), 7.06(H-4), 6.64(H-6), 2.93(H-10); **Gal:** 3.62(H-5), 3.34(H-3), 3.85(H-4), 3.56(H-6); ^{13}C- NMR δ_{ppm} : **Serot**: 150.5(C-5), 52.4(C-10), 23.4(C-9); **Gal**: 95.8(C-1), 60.6(C-6); **6-*O*-aryl-α-D-galactoside (8b) :** ^{1}H-NMR δ_{ppm}: **Serot:** 3.04(H-9); **Gal:** 3.68(H-2), 3.73(H-4), 3.52(H-6); ^{13}C-NMR δ_{ppm} **Serot**: 52.1(C-10), 23.7(C-9); **Gal**: 96.8(C-1), 60.7(C-6); **6-*O*-aryl-β-D-galactoside (8c)**: ^{1}H-NMR δ_{ppm}: **Serot:** 3.28(H-9); **Glu:** 3.65(H-4), 3.42(H-6); ^{13}C- NMR δ_{ppm} **Serot:** 52.4(C-10), 23.4(C-9); **Gal:** 97.7(C-1), 64.9(C-6).

Serotonyl 5-*O*-mannosides 9a-c: Solid; Isolated yield- 40.1mg (11.8%); UV(H_2O,λ_{max}): 210 nm($\sigma\rightarrow\sigma^*$,$\epsilon_{210.0}$ - 9641 M^{-1}), 274nm($n\rightarrow\pi^*$,$\epsilon_{274.0}$ -2754 M^{-1}), 367nm ($n\rightarrow\pi^*$,$\epsilon_{367.0}$-1573 M^{-1}); IR(stretching frequency, cm^{-1}), 1026(C-O-C glycosidic aryl alkyl asymmetrical), 1202(C-O-C glycosidic aryl alkyl symmetric), 1462(aromatic C=C), 3534(OH); MS (*m/z*) 337 $[M-1]^+$; 2D HSQCT (DMSO-d_6): **C1-β-D-mannoside (9a):** ^{1}H-NMR δ_{ppm}: (500.13 mhz): **Serot**: 7.08(H-3), 6.65(H-6); **Mann:** 3.49(H-2), 3.82(H-3), 3.62(H-4), 3.85(H-6); ^{13}C-NMR δ_{ppm}: **Serot:** 52.8(C-10), 23.9(C-9); **Mann**: 102.1(C-1), 75.6(C-2), 83.0(C-3), 70(C-4), 78.4(C-5), 64.0(C-6); **C6-*O*-ary-α-D-mannoside (9b):** ^{1}H-NMRδ_{ppm}: **Serot:** 6.94(H-6),3.20(H-9); **Mann** 3.52(H-4), 3.42(H-6); ^{13}C-NMR δ_{ppm} **Serot**: 51.2(C-10); **Glu**: 94.1(C-1), 81.9(C-3), 61.7(C-6); **C6-*O*-aryl-β-D-mannoside (9c)**: ^{1}H-NMR δ_{ppm}: **Serot:** 6.6(H-6), 3.14(H-9); **Mann:** 3.58(H-2), 3.69(H-3), 3.82(H-4, 3.89(H-6); ^{13}C- NMR δ_{ppm}: **Serot:** 52.2(C-10); **Mann:** 99.6 (C-1), 73.3(C-2), 82.6(C-3), 70.7(C-4), 62.7(C-6).

Serotonyl 5-*O*-ribosides 10a,b: Solid; Isolated yield- 60.5mg(19.6%); UV(H_2O, λ_{max}): 205.0nm($\sigma\rightarrow\sigma^*$,$\epsilon_{205.0}$ - 4170 M^{-1}), 276.0nm($n\rightarrow\pi^*$,$\epsilon_{276.0}$ - 1321M^{-1}); IR(stretching frequency, cm^{-1}): 3309(OH), , 1088(C-O-C glycosidic aryl alkyl symmetrical), 1208(C-O-C glycosidic aryl alkyl asymmetrical), 1458(aromaticc=C); MS(*m/z*)-310.04 $[M+2]^+$; 2D-HSQCT(DMSO-d_6): **C1-α-D-riboside (10a):** ^{1}H-NMR δ_{ppm}: **Serot:** 4.24(H-9), 3.12(H-10); **Ribo:** 3.72(H-2), 3.69(H-4), 3.90(H-6); ^{13}C-NMR δ_{ppm}: **Serot**: 150.9(C-5), 128.5(C-4), 53.9(C-10), 21.6(C-9); **Ribo**: 103.1(C-1),72.6(C-4), 71.2(C-2); **C1-β-D-riboside (10b):** ^{1}H-NMR δ_{ppm}: **Serot**: 6.78(H-3), 7.10(H-8), 3.25(H-9), 2.95(H-10); **Ribo:** 6.86(H-2), 3.94(H-3), 3.50(H-4), 3.65(H-6); ^{13}C-NMR δ_{ppm}: **Serot:** 50.2(C-10), 22.9(C-9); **Ribo**: 103.3(C-1), 73.4(C-4), 72.2(C-2).

Epinephryl-3-*O*/4-*O*—glucosides 11a,b: Solid; Isolated yield- 62.1mg (14.7%); UV(H_2O, λ_{max}): 209 nm ($\sigma\rightarrow \pi^*$, $\epsilon_{209.0}$ -5885.0M^{-1}), 266 nm ($\pi\rightarrow\pi^*$, - $\epsilon_{266.0}$ 9757.0M^{-1}), IR(stretching frequency cm^{-1}), 1212(glycosidic aryl alkyl C-O-C asymmetric), 1048(glycosidic aryl alkyl C-O-C asymmetric), 1459(C=C), 3190(OH); MS(*m/z*)-347.04 $[M-2]^+$; 2D-HSQCT (DMSO-d_6): **3-*O*-C1-β-D-glucoside (11a) :** ^{1}H-NMR δ_{ppm}: (500.13 mhz): **Epi:** 6.70(H-2), 6.51(H-5), 6.60(H-6) ; **Glu** 4.31(H-1) , 3.82(H-5), 3.60(H-3), 4.80(H-4), ^{13}C-NMR δ_{ppm}: **Epi**: 145.1(C-3), 144.8(C-4), 117.4(C-6), 115.3(C-5), 114.1(C-2), 131.8(C-1), 60.5(C-8); **Glu**: 97.5(C-1), 72.5(C-5), 71.1(C-4), 63.2(C-6); **4-*O*-C1-β-D-glucoside (11b):** ^{1}H-NMR δ_{ppm}: **Epi:** 6.76(H-2); **Glu** 4.30(H-1), 2.93(H-4), 3.24(H-5); ^{13}C-NMR δ_{ppm}: **Epi**: 145.1(C-3), 144.8(C-4), 117.4(C-6), 115.3(C-5), 114.1(C-2), 131.8(C-1), 60.5(C-8); **Glu**: 97.2(C-1), 82.1(C-3), 75.6(C-2), 70.4(C-4), 61.8(C-6).

Epinephryl-3-*O*/4-*O*—mannosides 12a-c: Solid; Isolated yield- 82.1mg (23.7%); UV(H_2O, λ_{max}): 231nm ($\sigma\rightarrow \pi^*$,$\epsilon_{231.0}$ - 92020 M^{-1}), 285nm($n\rightarrow\pi^*$,$\epsilon_{285.0}$ – 8390 M^{-1}). IR(stretching frequency, cm^{-1}), 1021(C-O-C glycosidic aryl alkyl asymmetrical), 1059(C-O-C glycosidic aryl alkyl asymmetrical), 1223(C-O-C glycosidic aryl alkyl symmetric), 1421(aromatic C=C), 3343(OH); MS (*m/z*) 364 $[M+1]^+$; 2D HSQCT (DMSO-d_6): **3-*O*-C1-β-D-mannoside (12a):** ^{1}H-NMR δ_{ppm}: (500.13 mhz): **Epi:** 3.35(H-7), 4.52(H-8); **Glu:** 4.34(H-1), 3.35(H-2), 3.90(H-6); ^{13}C-NMR δ_{ppm} **Epi:** 145.2(C-3), 144.6(C-4), 117.4(C-6), 115.1(C-5), 114.1(C-2), 53.4(C-8), 34.9(C-9); **Mann**: 94.2(C-1), 62.0(C-6); **4-*O*-C1-α-D-mannoside (12b):** ^{1}H-NMR δ_{ppm}: **Epi:** 3.42(H-7); **Mann** 4.85(H-1), 3.51(H-4), 3.54(H-6), ^{13}C-NMR δ_{ppm}: **Epi:** 145.2(C-3), 144.6(C-4), 117.4(C-6), 115.1(C-5), 114.1(C-2), 53.4(C-8), 34.9(C-9); **Mann**: 92.2(C-1), 63.2(C-6); **4-*O*-C1-β-D-mannoside (12c):** ^{1}H-NMR δ_{ppm}: **Epi:** 3.20(H-7); **Mann:** 4.85(H-1), 3.40(H-4), 3.53(H-6); ^{13}C-NMR δ_{ppm}: **Epi**: 145.2(C-3), 144.6(C-4), 117.4(C-6), 115.1(C-5), 114.1(C-2), 53.4(C-8), 34.9(C-9);**Mann**: 97.1(C-1), 64.1(C-6).

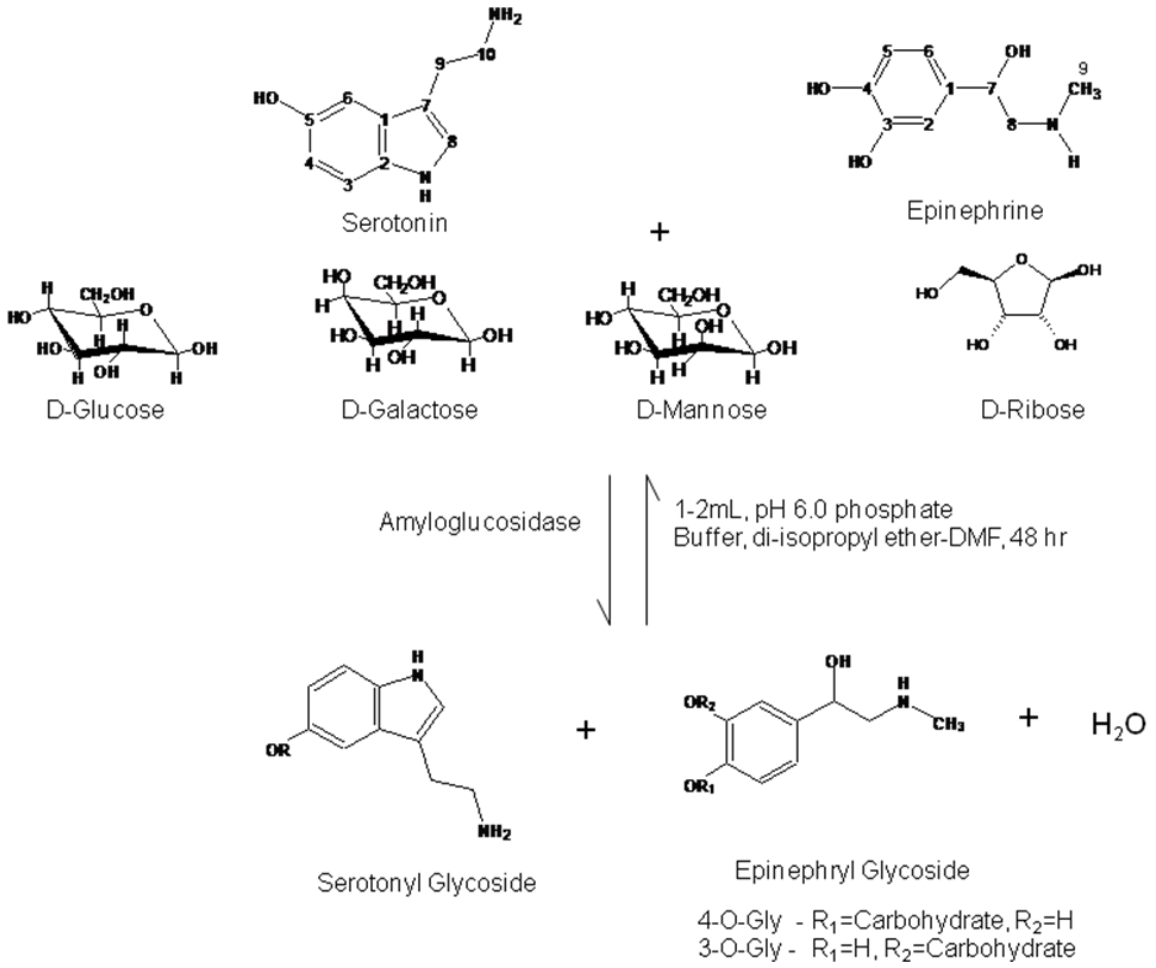
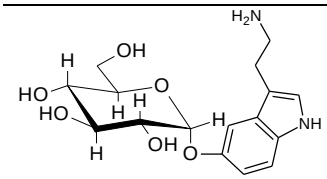
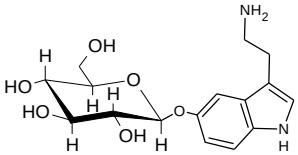
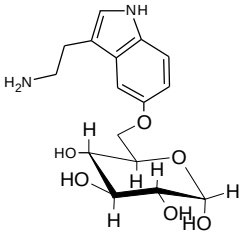
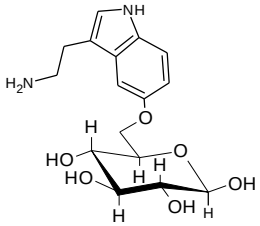
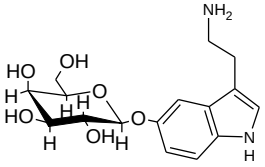
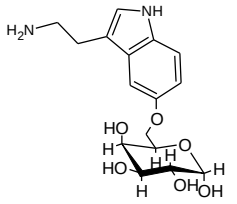
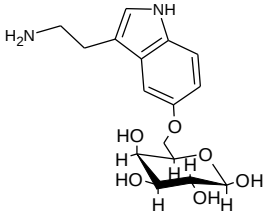
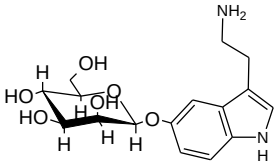
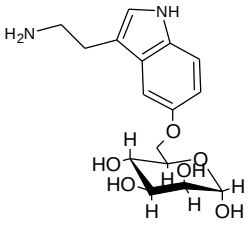
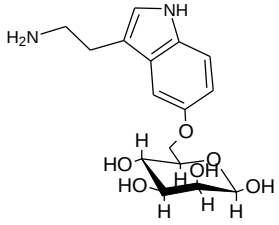
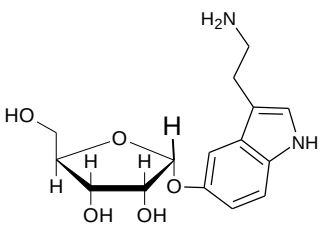
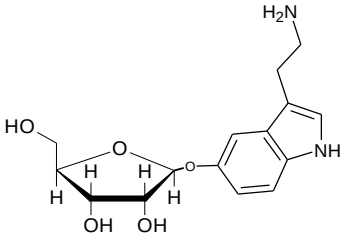


Figure 1. Synthesis of serotonyl and epinephryl glycosides

Table 1. Amyloglucosidase catalyzed syntheses of serotonin and epinephrine glycosides

Glycoside	Product (% Proportion) [a]	Yield (%) [b]
 **7a**: Serotonyl-5-*O*-(α-D-glucopyranoside)  **7b**: Serotonyl-5-*O*-(β-D-glucopyranoside)	C1 α (26), C1 β (39), 6-*O*-α (17) and 6-*O*-β (19) mono-glycosides	15
 **7c**: Serotonyl-5-*O*-(6-α-D-glucopyranoside)  **7d** Serotonyl-5-*O*-(6-β-D-glucopyranoside)		
 **8a**: Serotonyl-5-*O*(-β-D-galacto Pyranoside)  **8b**: Serotonyl-5-*O*-(6-α-D-galacto pyranoside)  **8c**: Serotonyl-5-*O*-(6-β-D-galacto pyranoside)	C1α (49) and 6- *O*-α (25), 6-*O*-β (26) mono-galactoside	13
 **9a** Serotonyl-5-*O*(-β-D-mannopyranoside)  **9b**: Serotonyl-5-*O*-(6-α-D-mannopyranoside)  **9c**: Serotonyl-5-*O*-(6-β-D-Mannopyranoside)	C1β (48) and 6-*O*-α (22) and 6- *O*-β (20) mono-mannoside	18
 **10a**: Serotonyl-5-*O*(-α-D-ribofuranoside)  **10b**: Serotonyl-5-*O*(-β-D-ribofuranoside)	C1α (72) and C1β (28) mono- riboside	29

RESULTS AND DISCUSSION

The reaction did not take place in case of both serotonin **1** and epinephrine **2** without the use of the enzyme amyloglucosidase from the *Rhizopus* mold under the reaction conditions employed. Reaction conditions employed for both serotonin and epinephrine are 1 mmole each of serotonin / epinephrine, 1mmole of carbohydrate, 40% w/w of amyloglucoidase to carbohydrate, 2 ml of 0.01 M ph 6.0 phosphate buffer in 100 ml diisopropyl ether under reflux at 68^{o}c for a period of 48h.

Both serotonin and epinephrine contain phenolic –OH groups. Serotonin (**Fig. 1**) contains phenolic –OH group at position 5. Epinephrine molecule contains two phenolic groups at position 3 and 4 and one secondary alcohol group at position 7 (**Fig. 1**), all of which are capable of undergoing glycosylation. The carbohydrates employed were D-glucose **3**, D-galactose **4**, D-mannose **5**, D-ribose **6**, D-arabinose, D-fructose, maltose, lactose, sucrose, D-sorbitol and D-mannitol

Serotonyl 5-O-glycosides

In case of serotonin, reaction took place with D-glucose **3**, D-galactose **4**, D-mannose **5** and D-ribose **6** only. The other carbohydrate molecules did not react. This could be due to competetive binding of serotonin and carbohydrates to the enzyme restricting reactions with certain carbohydrates as fascile transfer of tightly bound serotonin to the lesser nucleophilic carbohydrates rendered impossible. Glycosylation yields were generally in the range 13-29% (Table 1). Reaction profile was studied as a function of the reaction period for the enzyme catalysed reaction with D-ribose. The yields evaluated at different periods by HPLC are : 12h -10%, 24h -18%, 36h – 23h and 48h – 29%.

UV spectra of serotonyl glycosides showed uv_{max} at 205 to 215nm (σ-σ* transition), 274 to 296nm (n-π* transition) and 367nm for extended n-π* transition (270nm for free serotonin). IR C-O-C glycosidic aryl alkyl symmetrical stretching frequencies in the 1016-1088 cm^{-1} range and assymmetrical stretching frequencies in the 1200-1208 cm^{-1} range indicated that serotonin has undergone glycosylation. Mass spectra also confirmed glycosylation through detection of the parent M^{+} ion for the glycosides. From 2DHSQCT spectra, the following glycosydic formation were confirmed from the respective chemical shift values: from D-glucose **3,** C1-α-glucoside (**7a**) to C1- α at 95.9 ppm, C1-β-glucoside (**7b**) to C1- β at 101.8 ppm, 6-O-aryl- α-D-glucose (**7c**) to C6- α at 62.7 ppm and H6 -α at 3.58ppm and 6-O-aryl- β-D-glucose (**7d**) to C6- β at 62.6ppm and H6- β at 3.86ppm; from D-galactose **4**, C1-β-galactoside (**8a**) to C1-β at 95.8ppm, C6-O-arylated-D-galactoside (**8b**) to C1-α at 96.8ppm, C6 at 60.7ppm and H-6a at 3.52ppm, C6-O-aryl- β-D-galactoside (**8c**) to C1-β at 97.7ppm, C6 at 64.9ppm, H6-β at 3.42ppm, from D-mannose **5**, C1-β-mannoside (**9a**), to C1 β at 102.1ppm, C6- α-mannoside (**9b**) to C1-α at 94.1 ppm; C6-O-aryl-β-D-mannoside (**9c**) to C1-β at 99.6ppm, C6 at 62.7 ppm, and H-6a at 3.89ppm; from D-ribose **6**, C1-α-riboside (**10a**) to C1-α at 103.1ppm and C1-β-riboside (**10b**) to C1-β at 103.3ppm. This was also reflected in the respective chemical shift values of the phenolic hydroxylic carbon at position 5 of serotonin at around 150ppm.

Except for the serotonyl glucosides, all the other glycosides and 6-O-derivatives are being reported for the first time. Since the phenolic group at position 5 in serotonin is not sterically hindered, all the four carbohydrates D-glucose **3**, D-galactose **4**, D-mannose **5** and D-ribose **6** underwent fascile reaction with serotonin.

Epinephrinyl glycoside

In case of epinephrine, out of the 11 carbohydrates employed only D-glucose **3** and D-mannose 5 reacted effectively. The other carbohydrates molecules did not react. Structural comparison between serotonin and epinephrine clearly shows that epinephrine is more polar compared to serotonin and hence could bind much more strongly to the enzyme compared to serotonin, inhibiting the enzyme towards reaction with other carbohydrate molecules. Compared to the other carbohydrate molecules besides D-glucose and D-mannose, epinephrine could bind more strongly than those other carbohydrate molecules thereby inhibiting reaction.

While the reaction with glucose exhibited a conversion yield of 18%, D-mannose **5** gave only 29% yield. Both 3-O- and 4-O- products were formed. Only phenolic –OH groups of epinephrine at position 3 and 4 reacted in a fascile manner. No product formation arising out of reaction at the secondary hydroxyl group at position 7 was detected. Reaction profile was studied as a function of the reaction period for the enzyme catalysed reaction with D-glucose. The yields evaluated at different periods by HPLC are : 10h -6%, 20h -10%, 34h – 15% and 48h – 18%.

UV spectra showed λ_{max} at 208nm and 231nm (σ- σ* transition) for the reaction products of D-glucose **3** and D-mannose **5** repectively and another λ_{max} at 266nm for D-glucoside and 285 nm (n-π* transition) for D-mannoside (279nm for epinephrine itself). Similarly IR, glycosidic aryl-alkyl C-O-C stretching frequencies at 1048cm^{-1} for D-glucoside and 1021cm^{-1} for D-mannoside and assymmetrical stretching frquencies at 1212cm^{-1} and 1223cm^{-1} for the respective

glucoside and mannosides indicated glycosylation. 2DHSQCT spectra confirmed the product formation further from the respective chemical shift values: from D-glucose **3**, epinephryl 3-*O*-C1-β-D-glucoside **(11a)** to C1β at 97.5ppm and H-1 β at 4.31ppm and epinephryl 4-*O*-C1-β-D-glucoside **(11b)**, to C1β at 97.2ppm and H-1 β at 4.30ppm, for D-mannose **5**, epinephryl 3-*O*-C1-β-D-mannoside **(12a)** to C1β at 94.2ppm and H-1β at 4.34ppm, epinephryl 4-*O*-C1-α-D mannoside **(12b)**, C1α at 92.2ppm and H-1α at 4.85 and epinephryl 4-*O*-C1-β-D mannoside (**12c)** to C1β at 97.1ppm and H-1 β at 4.85ppm. Further the C3 and C4 carbons of epinephrine showed shifts at around 144-145 ppm in their respective derivatives indicating reaction involving these phenolic hydroxyl groups.

In both serotonin and epinephrine, the reaction did not occur at the primary and secondary amino groups respectively. Epinephrine formed only monoglycosylated derivatives although three –OH groups were available for glycosylation. Presence of hydrophobic/hydrophilic phenolic –OH group of serotonin and epinephrine bestowed excellent nucleophilicity to these molecules promoting reaction with few diverse carbohydrate molecules. Only mono glycosylated/arylated products were detected. However, loss of regiospecificity in case of epinephrine could be due to employemnet of large concentration of the enzyme. Reaction with only few carbohydrate molecules indicated that both serotonin and epinephrine possess the propensity to inhibit the enzyme strongly. An attempt to separate the glycosides through column chromatography on Bio-Gel P2 was not successful due to similar molecular weights. Though we detected higher solubilities of the mixture of the glycosides than their respective aglycons, the solubilities of individual glycosides could not be determined because of the above mentioned problem. Thus the solubilities of serotonyl 5-O-glucosides **7a-d** and that of epinephryl 3-O/4-O-D-glucosides 11**a,b** were found to be 7.6g/100ml and 4.6g/100ml at 25^{o}C respectively. The higher solubilities could portend excellent pharmaceutical properties for the prepared glycosides.

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