Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 339 (2004) 1517–1529
Carbohydrate RESEARCH

Enzymatic synthesis of two salicin analogues by reaction of salicyl alcohol with *Bacillus macerans* cyclomaltodextrin glucanyltransferase and *Leuconostoc mesenteroides* B-742CB dextransucrase

Seung-Heon Yoon,[a,b] D. Bruce Fulton[b] and John F. Robyt[a,b,*]

[a]*Laboratory of Carbohydrate Chemistry and Enzymology, Iowa State University, Ames, IA 50011, USA*
[b]*Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, IA 50011, USA*

Received 25 August 2003; accepted 9 March 2004

Abstract—β-Salicin is a naturally occurring glycoside found in the bark of poplar and willow trees. Ancient man used it as an analgesic and antipyretic. It has a D-glucopyranose unit attached by a β-linkage to the phenolic hydroxyl of salicyl alcohol. Two new salicin analogues have been enzymatically synthesized by transglycosylation reactions: (*a*) by the reaction of *Bacillus macerans* cyclomaltodextrin glucanyltransferase with cyclomaltohexaose and salicyl alcohol, followed by reactions with alpha amylase and glucoamylase to give D-glucopyranose attached by an α-linkage to the phenolic hydroxyl of salicyl alcohol as the major product, α-salicin; and (*b*) by the reaction of *Leuconostoc mesenteroides* B-742CB dextransucrase with sucrose and salicyl alcohol, followed by reactions with dextranase and glucoamylase to give α-D-glucopyranose attached to the primary alcohol hydroxyl of salicyl alcohol as the major product, α-isosalicin.

Keywords: Salicyl alcohol; Salicin analogues; α-Salicin; α-Isosalicin; β-Salicin; *Bacillus macerans* cyclomaltodextrin glucanyltransferase; Cyclomaltohexaose; Dextransucrase

1. Introduction

Many of the naturally occurring glycosides found in plants have biological activities.[1–7] The activities are primarily due to the aglycone of the glycosides. The water solubility of hydrophobic molecules, containing one or more hydroxyl groups, can be increased by glycosylation, which can influence their physicochemical and pharmacological properties, such as transport through membrane barriers and transport by body fluids.[2] Glycosylation also often reduces the irritability and toxicity of the aglycone.[2]

Salicin [2-(hydroxymethyl)phenyl-β-D-glucopyranoside] is a plant glycoside found in poplar (*Populus*) and willow (*Salix*) barks. It was used by ancient man as an anti-inflammatory, analgesic, and antipyretic prodrug.[3,8]

Bacillus macerans cyclomaltodextrin glucanyltransferase (CGTase) catalyzes transglycosylation reactions between cyclomaltohexaose and acceptors having a hydroxyl group.[9] It also catalyzes disproportion reactions between two maltodextrin chains.[9] *Leuconostoc mesenteroides* B-742CB dextransucrase catalyzes the transfer of D-glucose from sucrose primarily to a primary alcohol hydroxyl of various carbohydrate acceptors to give new dextrins.[10]

In the present study, we report two distinct glycosylation reactions of salicyl alcohol. In the first reaction, *B. macerans* CGTase transferred maltohexaose from cyclomaltohexaose to the primary alcohol hydroxyl of salicyl alcohol. This was followed by treatments with alpha amylase, glucoamylase, and immobilized yeast

* Corresponding author. Tel.: +1-515-294-1964; fax: +1-515-294-0453; e-mail: jrobyt@iastate.edu

0008-6215/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carres.2004.03.018

to give a new salicin derivative, α-salicin, with glucose attached to the phenolic hydroxyl group. In the second reaction, *L. mesenteroides* B-742CB dextransucrase transferred glucose from sucrose to the primary hydroxyl group of salicyl alcohol. This was followed by treatments with dextranase and glucoamylase to give a second new salicin derivative, α-isosalicin, with glucose attached to the primary alcohol group of salicyl alcohol.

2. Experimental

2.1. Materials

Salicyl alcohol was obtained from Aldrich (Milwaukee, WI). *Bacillus macerans* CGTase [EC 2.4.1.19] was obtained by growing *B. macerans* ATCC 8517 on wheat bran media and purification by a modification of the method of Kobayashi et al.[11] as previously described.[12] Porcine pancreatic alpha amylase (PPA) was obtained from Boehringer Mannheim (Indianapolis, IN). *Aspergillus niger* glucoamylase (GA) was obtained from Megazyme International (Wicklow, Ireland). Dextransucrase [EC 2.4.1.5] from *L. mesenteroides* B-742CB was prepared in our laboratory by previously reported procedures.[13,14] Dextranase from *Penicillium* sp. was obtained from Sigma (St. Louis, MO). Cyclomaltohexaose (α-CD) was obtained from Ensuiko Sugar Refining Co. (Yokohama, Japan). Immobilized *Saccharomyces cerevisiae* was prepared by the procedure previously described.[15]

2.2. Preparation of salicyl alcohol analogues by reaction with CGTase

B. macerans CGTase (240 IU, 1 IU is the amount of enzyme that will produce 1 μmol of cyclomaltodextrin per min at pH 6.0 and 37 °C) was added to 12 mL of substrate solution, containing 100 mM α-CD and 100 mM salicyl alcohol in 25 mM imidazolium HCl buffer (pH 6.0). The enzyme reaction was carried out at 37 °C for 72 h with stirring, and the reaction was stopped by heating in boiling water for 5 min. Porcine pancreatic alpha amylase (PPA, 120 IU, 1 IU is that amount of enzyme that will hydrolyze 1 μmol of α-(1 → 4) glucosidic linkages per min at pH 6.0 and 37 °C) and glucoamylase (GA, 120 IU, 1 IU is the amount of enzyme that will produce 1 μmol of glucose from waxy maize starch per min at pH 6.0 and 37 °C) were added to 12 mL of the CGTase reaction mixture and incubated at 37 °C for 24 h. These two enzymes were used to produce glucosyl salicyl alcohol from maltodextrin conjugated salicyl alcohol by hydrolyzing the maltodextrins. The PPA and GA enzyme reactions were stopped by heating in boiling water for 5 min and cooled to 21 °C. Immobilized yeast was used to remove fermentable saccharides,[15] which were produced as by-products from the enzyme reactions. Wet immobilized yeast (18 g) was added to the reaction digest and the mixture was kept at 37 °C for 24 h. The immobilized yeast was removed by filtration, and washed three times with 6 mL each of deionized water. The washings were added to the supernatants of the digests. The supernatants were then concentrated to about 2 mL by vacuum rotary evaporation. The insoluble material in the concentrated solution was removed by centrifugation at 4400*g* for 10 min. The reaction products were analyzed by TLC. An appropriate amount (1–5 μL) of sample was loaded onto a 10 × 20 cm Whatman K5 silica gel plate (Fisher Scientific, Chicago, IL). The plate was irrigated two times with 85:15 volume parts of acetonitrile–water at 21 °C, with an 18 cm irrigation path length. The carbohydrates on the TLC plate were visualized by dipping the plate into a methanol solution containing 0.3% (w/v) *N*-(1-naphthyl)ethylenediamine and 5% (v/v) sulfuric acid, followed by heating at 120 °C for 10 min.[16] The relative percents of the carbohydrates were determined by TLC densitometry, using a Bio-Rad scanning densitometer (GS-670, Bio-Rad Laboratories, Hercules, CA).[16,17]

2.3. Preparation of salicyl alcohol analogues by reaction with dextransucrase

L. mesenteroides B-742CB dextransucrase [EC 2.4.1.5] (12 IU) was added to 12 mL of substrate solution, containing 200 mM sucrose and 100 mM salicyl alcohol in 20 mM pyridinium acetate buffer (pH 5.2). The enzyme reaction was carried out at 25 °C for 24 h with magnetic stirring, and was stopped by heating in boiling water for 5 min. Dextranase (DA, 120 units) and glucoamylase (GA, 120 units) were added to 12 mL of the dextransucrase reaction mixture and incubated at 37 °C for 24 h. DA and GA were used to increase the amount of glucosyl salicyl alcohol by hydrolyzing isomaltodextrins that were conjugated to salicyl alcohol. The DA and GA enzyme reactions were stopped by heating in boiling water for 5 min and cooled down to 20 °C. Immobilized yeast (18 g) was used as described above. The supernatants were concentrated to about 2.4 mL by vacuum rotary evaporation. Insoluble dextran in the concentrated reaction mixture was removed by centrifugation at 9000*g* for 10 min. The enzyme reaction products were analyzed by TLC as described above.

2.4. Fractionation of the enzyme products by BioGel P2 column chromatography

About 0.6–0.8 mL of the concentrated digests were loaded onto BioGel P2 (fine) column (1.5 × 115 cm), and eluted with deionized water at a flow rate of 3.78 mL/h,

collecting 1.0 mL fractions. This procedure was repeated several times to fractionate the entire concentrated reaction digest. The carbohydrate content of each fraction was determined by the micro phenol–H_2SO_4 method,[18] and the composition of the fractions were analyzed by TLC as described above.

2.5. Purification of the enzyme products by descending paper chromatography

Each of the reaction products obtained from the BioGel P2 column chromatography were further purified by preparative descending paper chromatography:[19] an appropriate amount (50–180 μL) of the reaction products (pooled fractions 1–7) were loaded onto Whatman 3 mm paper (23 × 56 cm). The paper was irrigated with 10:4:3 volume parts of EtOAc–pyridine–water for 5.5–36 h. Strips (2.54 cm) were cut on both sides of the paper and the products on the paper were visualized by the silver nitrate method and used to locate the products,[19] which were cut from the paper, eluted with deionized water, and concentrated to about 0.5 mL by vacuum rotary evaporation for further study.

2.6. Analysis of the products by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS)

Salicyl alcohol (1 μL, 1 mg/mL) and the purified reaction products (about 1 mg/mL) were transferred to the probe, and mixed with 1.0 μL of 0.1 M 2,5-dihydroxybenzoic acid (DHB) in 70% (v/v) acetonitrile. The solvent was evaporated under a stream of cold air or by vacuum. The masses of the samples were analyzed by MALDI-TOF MS,[13,20] using a Dynamo instrument (Thermo Bio-Analysis, Ltd, Paradise, UK) with a nitrogen laser (337 nm). Ions were detected in a positive mode at an acceleration voltage of 20 kV.

2.7. NMR analysis of the products

About 50 mg of salicyl alcohol and 5–50 mg of the purified products were exchanged three to four times with 600 μL of D_2O and then dissolved in 600 μL of pure D_2O, and placed into 5 mm NMR tubes. NMR spectra were obtained on a Bruker DRX 500 spectrometer, operating at 500 MHz for ^1H and 125 MHz for ^{13}C at 25 °C. COSY (homonuclear correlation spectroscopy) and HMQC (heteronuclear multiple quantum coherence spectroscopy) spectra were recorded, following standard experimental protocols, and analyzed with the XWINNMR (Bruker) program.[13] Sodium 4,4-dimethyl-4-sila propanesulfonate (DDS) was used as a ^1H-chemical shift standard and 1,4-dioxane was used as the ^{13}C standard.

3. Results

3.1. Reaction of CGTase with cyclomaltohexaose and salicyl alcohol followed by alpha amylase and glucoamylase

B. macerans CGTase catalyzed a transglycosylation reaction between salicyl alcohol and cyclomaltohexaose, giving maltohexaosyl salicyl alcohol. This was followed by a series of transglycosylation reactions (disproportionation reactions) between two maltodextrin units attached to two salicyl alcohol molecules to give several salicyl alcohol analogues with D-glucose and maltodextrins of different sizes attached to salicyl alcohol (Fig. 1A, lane 3). When PPA and GA were added to these products, several CGTase reaction products (PC3, PC4, PC6, PC7, PC8, PC9, maltodextrins, and higher molecular weight products) were removed or significantly decreased, while the amounts of PC1, PC2, and D-glucose were increased and new products (PC5, PC10, PC12, and PC13) were produced (Fig. 1A, lane 4). Table 1 gives the relative percents of the compounds in the reaction digest obtained by TLC determinations. When the PPA and GA treated products were treated with immobilized yeast, most of the free D-glucose was removed and PC2 resulted as the major product (Fig. 1A, lane 5 and Table 1).

3.2. Reaction of *L. mesenteroides* B-742CB dextransucrase with sucrose and salicyl alcohol followed by dextranase and glucoamylase

L. mesenteroides B-742CB dextransucrase catalyzed the transfer of D-glucose from sucrose to salicyl alcohol to produce several salicyl alcohol analogues, D-fructose, D-glucose, leucrose, and isomaltodextrins (Fig. 1B, lane 2). When the dextransucrase reaction was treated with dextranase and glucoamylase, several products (PD7, PD8, PD10, and high molecular weight products) were significantly or completely removed, and at the same time the relative amounts of PD1, PD2, PD3, PD5, D-glucose, and isomaltose were increased (Fig. 1B, lane 3, and Table 2). When the dextranase- and glucoamylase-treated digest was incubated with immobilized yeast, the fermentable saccharides, mainly D-glucose and D-fructose, were removed (Fig. 1B, lane 4, Table 2).

3.3. Purification of the reaction products on BioGel P2

The immobilized yeast-treated CGTase reaction products were fractionated into seven groups by BioGel P2 gel permeation column chromatography (data not shown). PC4 was a single compound on TLC, but was resolved into two compounds ($PC4_1$ and $PC4_2$) on the BioGel P2 column. The compounds that were

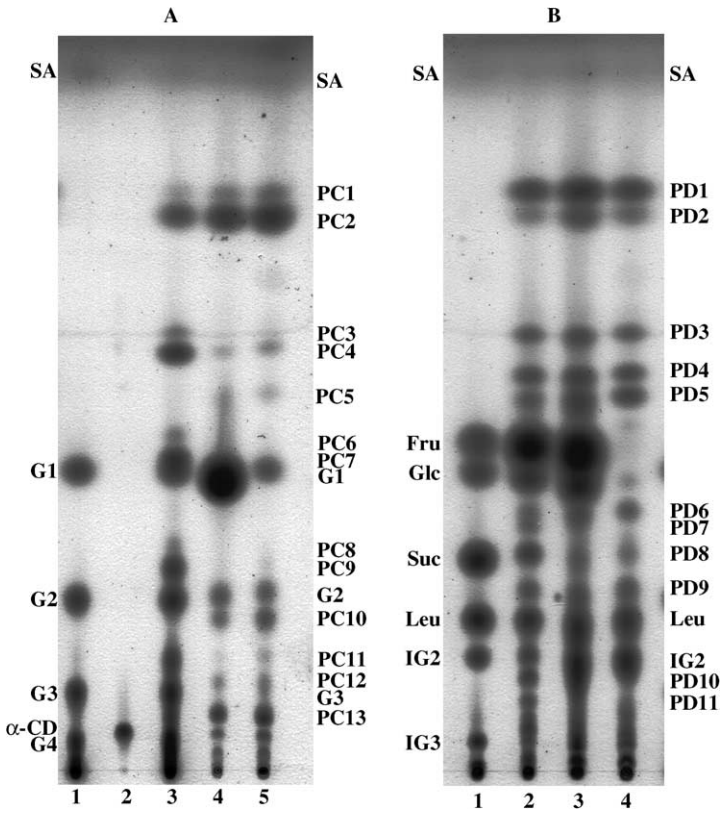

Figure 1. Thin-layer chromatogram of the cyclomaltodextrin glucanyltransferase (CGTase) reaction products from salicyl alcohol (SA) and cyclomaltohexaose (α-CD) (A) and dextransucrase B-742CB reaction products from SA and sucrose (B). (A) Lane 1, maltodextrin standard; lane 2, α-CD; lane 3, CGTase reaction products before porcine pancreatic alpha amylase (PPA) and glucoamylase (GA) treatment; lane 4, CGTase reaction products after PPA and GA treatment; lane 5, enzyme reaction products after immobilized yeast treatment. G_n denotes maltodextrins having n number of D-glucose units. (B) Lane 1, salicyl alcohol and carbohydrate standards; lane 2, dextransucrase reaction products before dextranase (DA) and glucoamylase (GA) treatment; lane 3, dextransucrase reaction products after DA and GA treatment; lane 4, enzyme reaction products after immobilized yeast treatment. IG_n denotes isomaltodextrins having n units of D-glucose.

Table 1. The relative percent of the products from the CGTase reaction of cyclomaltohexaose with salicyl alcohol

Reaction products[b]	Rxn 1[a] *w* CGTase	Rxn 2[a] PPA[c] + GA[c]	Rxn 3[a] ImYeast
PC1	1.07	3.03	6.21
PC2	6.26	12.69	31.24
PC3	1.54	0	0
PC4	7.89	1.04	2.25
PC5	0	1.98	1.78
PC6	2.10	0	0
PC7	Tr	Tr	0
G_1	14.93	64.5	11.94
PC8	1.25	0	0
PC9	6.27	0	0
G_2	11.43	3.76	6.09
PC10	0	2.17	6.73
PC11	6.98	Tr	1.58
PC12	0	1.04	3.17
G_3	9.90	0	0
PC13	0	3.94	8.68
HMWP[b]	30.38	5.87	21.1

[a] Salicyl alcohol is not included; Rxn 1, CGTase reaction products from 100 mM salicyl alcohol and 100 mM cyclomaltohexaose; Rxn 2 is Rxn 1 + PPA + GA; Rxn 3 is Rxn 2 treated with immobilized yeast.
[b] PC = CGTase reaction product; G_1, G_2, G_3 = D-glucose, maltose, and maltotriose; HMWP = high molecular weight products; Tr = trace.
[c] PPA = porcine pancreatic alpha amylase; GA = glucoamylase.

Table 2. The relative percent of the products from the reaction of dextransucrase with sucrose and salicyl alcohol

Reaction products[b]	Rxn 1[a] *w* DS	Rxn 2[a] DA[c] + GA[c]	Rxn 3[a] ImYeast
PD1	4.94	8.07	10.35
PD2	2.58	3.87	4.72
PD3	3.12	3.92	4.65
PD4	3.82	3.72	5.58
PD5	3.37	4.70	7.23
Fructose	27.57	29.76	0
Glucose	9.14	10.9	0
PD6	2.12	2.8	4.61
PD7	2.44	0	0
PD8	5.49	2.61	3.43
PD9	3.49	2.82	5.19
Leucrose	7.82	7.43	14.32
Isomaltose	3.65	7.13	15.87
PD10	2.84	0	0
PD11	1.79	2.72	3.19
HMWP[b]	15.83	9.58	20.87

[a] Salicyl alcohol was not included; Rxn 1, dextransucrase (DS) reaction products from 100 mM salicyl alcohol and 200 mM sucrose; Rxn 2 is Rxn 1 + DA + GA; Rxn 3 is Rxn 2 treated with immobilized yeast.
[b] PD = dextransucrase reaction product; HMWP = high molecular weight products.
[c] DA = dextranase; GA = glucoamylase.

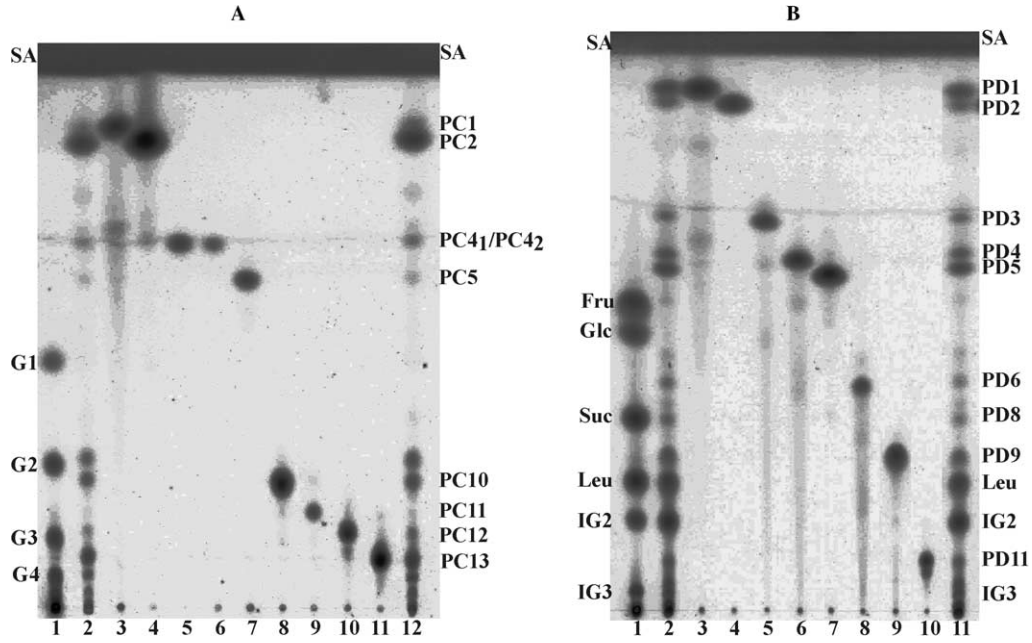

Figure 2. (A) Thin-layer chromatogram of the purified cyclomaltodextrin glucanyltransferase (CGTase) reaction products by BioGel P2 column chromatography and preparative descending paper chromatography. Lane 1, maltodextrin standard; lane 2 and 12, CGTase reaction products before purification; lane 3, PC1; lane 4, PC2; lane 5, PC4$_1$; lane 6, PC4$_2$; lane 7, PC5; lane 8, PC10; lane 9, PC11; lane 10, PC12; lane 11, PC13. G$_n$ denotes maltodextrins having *n* units of D-glucose. (B) Thin-layer chromatogram of the *L. mesenteroides* B-742CB dextransucrase reaction products after by BioGel P2 column chromatography and preparative descending paper chromatography. Lane 1, carbohydrate standards; lane 2 and 11, dextransucrase reaction products before purification; lane 3, PD1; lane 4, PD2; lane 5, PD3; lane 6, PD4; lane 7, PD5; lane 8, PD6; lane 9, PD9; lane 10, PD11. IG$_n$ denotes isomaltodextrins having *n* units of D-glucose.

fractionated on the BioGel P2 column were further purified by descending paper chromatography and all of the products were purified to single compounds (Fig. 2A).

The dextransucrase reaction products, remaining after yeast fermentation, were fractionated into nine groups by BioGel P2 column chromatography (data not shown). The fractionated products (groups 1–7) were further purified by preparative, descending paper chromatography. All reaction products except PD8 and PD11 were highly purified (Fig. 2B).

3.4. Determination of the molecular weights of the purified products by MALDI-TOF MS

The molecular weights of the CGTase and the dextransucrase reaction products were determined by mass spectrometry (MALDI-TOF MS). The MS-spectral results of the substrate, salicyl alcohol, and the major reaction products from the CGTase and dextransucrase reactions, PC1 and PD1, and PC2 and PD2, are given in Table 3. The purified products fell into two groups: (*a*) those with carbohydrate attached to salicyl alcohol

Table 3. The theoretical and MALDI-TOF MS determined molecular weights of salicyl alcohol and its synthesized salicyl alcohol analogues and the synthesized carbohydrates, resulting from the reaction of CGTase and dextransucrase with salicyl alcohol

Compound	Mass number (*m*/*z*)		CGTase reaction products	Dextransucrase reaction products
	Calculated[a] $[M+Na]^+$	Determined[b] $[M+Na]^+$		
Salicyl alcohol (SA)	124.1	124.2	—	—
Salicyl alcohol with one glucose unit (G_1–SA)	309.1	309.2	PC1, PC2	PD1, PD2
Salicyl alcohol with two glucose units (G_1–G_1–SA)	471.2	471.3	PC4$_1$, PC4$_2$, PC5	PD3, PD4, PD5
Salicyl alcohol with three glucose units (G_1–G_1–G_1–SA)	633.2	633.2	—	PD6
Disaccharide (G_1–G_1)	365.1	365.2	PC10, PC11	PD9
Trisaccharide (G_1–G_1–G_1)	527.2	527.2	PC12, PC13	—

[a] Mass numbers were calculated from C = 12.000, H = 1.0078, O = 15.9949, N = 14.0031, and Na = 22.9898; mass number of SA was from $[M]^+$.
[b] $[M+Na]^+$ and $[M]^+$ (for SA) were determined by MALDI-TOF MS.

Table 4. [1]H NMR chemical shifts[a] for salicyl alcohol and its analogues produced by the reaction of CGTase with cyclomaltohexaose and salicyl alcohol

	Position[b]	Salicyl alcohol	PC1[c]	PC2[c]	$PC4_1$[c]	$PC4_2$ [c]	PC5[c]
Salicyl alcohol (SA)	*H*-1	—	—	—	—	—	—
	H-2	—	—	—	—	—	—
	H-3	7.24	7.36	7.45	7.41	7.40	7.42
	H-4	6.89	6.96	7.07	7.12	7.13	7.14
	H-5	7.18	7.28	7.31	7.37	7.37	7.39
	H-6	6.86	6.94	7.19	7.24	7.25	7.25
	H-7	4.59	4.63	4.60	4.65	4.65	4.66
	H-7′	4.59	4.73	4.75	4.80	4.80	4.79
Glc(I)[d]	*H*-1		5.03	5.68	5.76	5.73	5.75
			(3.4)	(3.1)	(3.4)	(3.1)	(3.4)
	H-2		3.53	3.72	3.85	3.79	3.78
	H-3		3.69	3.95	4.10	4.24	3.96
	H-4		3.41	3.50	3.76	3.75	3.59
	H-5		3.67	3.68	3.79	3.83	3.92
	H-6		3.71	3.69	3.79	3.76	3.65
	H-6′		3.71	3.69	3.85	3.81	3.91
Glc(II)[e]	*H*-1				5.45	5.42	4.85
					(3.4)	(3.4)	(3.1)
	H-2				3.58	3.58	3.48
	H-3				3.77	3.68	3.63
	H-4				3.46	3.40	3.40
	H-5				4.06	3.69	3.68
	H-6				3.73	3.76	3.72
	H-6′				3.78	3.76	3.81

[a] Chemical shifts and coupling constants (in parentheses) were measured at 500 MHz in D_2O at 25 °C, using sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as a standard.
[b] Chemical structures and numbers refer to Figure 3.
[c] PC indicates a product from the reaction; PC1, product 1; PC2, product 2; and so forth.
[d] For the glucose unit attached to salicyl alcohol.
[e] For the glucose unit of the disaccharide attached to salicyl alcohol.

Table 5. [13]C NMR chemical shifts[a] for salicyl alcohol and its analogues produced by the reaction of CGTase with cyclomaltohexaose and salicyl alcohol

	Position[b]	Salicyl alcohol	PC1[c]	PC2[c]	$PC4_1$[c]	$PC4_2$[c]	PC5[c]
Salicyl alcohol (SA)	*C*-1	154.3	154.9	154.2	154.1	154.2	154.0
	C-2	126.7	124.0	129.7	129.7	129.8	129.7
	C-3	130.0	131.4	130.1	130.2	130.1	130.1
	C-4	121.0	121.0	123.3	120.2	123.3	123.4
	C-5	129.8	130.5	130.0	130.0	130.0	130.0
	C-6	115.8	116.2	115.4	115.2	115.3	115.5
	C-7	60.1	66.0	59.9	59.7	59.7	59.8
Glc(I)[d]	*C*-1		98.3	97.0	97.0	96.6	96.8
	C-2		71.8	71.8	70.2	71.5	71.6
	C-3		73.6	73.5	79.6	73.8	74.0
	C-4		69.9	69.8	70.2	77.1	70.0
	C-5		72.3	73.1	73.3	71.4	71.7
	C-6		60.7	60.8	60.7	60.8	65.9
Glc(II)[e]	*C*-1				99.4	100.2	98.2
	C-2				72.1	72.1	71.8
	C-3				72.9	73.2	73.6
	C-4				69.7	69.7	69.9
	C-5				72.1	73.0	72.3
	C-6				60.4	60.6	60.9

[a] Chemical shifts were measured at 125 MHz in D_2O at 25 °C, using 1,4-dioxane as a standard.
[b] Chemical structures and numbers refer to Figure 3.
[c] PC indicates products; PC1, product 1; PC2, product 2; and so forth.
[d] For the glucose unit attached to salicyl alcohol.
[e] For the second glucose unit of the disaccharide attached to salicyl alcohol.

Table 6. ^{1}H NMR chemical shifts[a] for salicyl alcohol and its analogues produced from the reaction of dextransucrase with sucrose and salicyl alcohol

	Position[b]	PD1[c]	PD2[c]	PD3[c]	PD4[c]	PD5[c]	PD6[c]
Salicyl alcohol (SA)	*H*-1	—	—	—	—	—	—
	H-2	—	—	—	—	—	—
	H-3	7.36	7.45	7.38	7.38	7.42	7.39
	H-4	6.96	7.07	6.96	6.95	7.14	6.99
	H-5	7.28	7.31	7.28	7.29	7.39	7.30
	H-6	6.94	7.19	6.93	6.93	7.25	6.94
	H-7	4.63	4.60	4.66	4.69	4.66	4.70
	H-7′	4.73	4.75	4.75	4.75	4.79	4.76
Glc(I)[d]	*H*-1	5.03	5.68	5.04	5.01	5.75	5.05
		(3.4)	(3.1)	(3.4)	(3.4)	(3.4)	(3.4)
	H-2	3.53	3.72	3.62	3.55	3.78	3.57
	H-3	3.69	3.95	3.82	3.67	3.96	3.68
	H-4	3.41	3.50	3.64	3.52	3.59	3.56
	H-5	3.67	3.68	3.69	3.84	3.92	3.81
	H-6	3.71	3.69	3.70	3.48	3.65	3.54
	H-6′	3.71	3.69		3.92	3.91	3.92
Glc(II)[e]	*H-1*			5.31	4.90	4.85	5.12
				(3.4)	(3.1)	(3.1)	(3.1)
	H-2			3.52	3.52	3.48	3.65
	H-3			3.72	3.72	3.63	3.83
	H-4			3.41	3.41	3.40	3.48
	H-5			3.97	3.69	3.68	3.72
	H-6			3.75	3.76	3.72	3.76
	H-6′			3.78	3.83	3.81	3.82
Glc(III)[f]	*H-1*						5.09
							(3.4)
	H-2						3.53
	H-3						3.75
	H-4						3.44
	H-5						3.89
	H-6						3.76
	H-6′						3.82

[a]Chemical shifts and coupling constants (in parentheses) were measured at 500 MHz in D_2O at 25 °C with sodium 3-(trimethylsilyl)-1-propane sulfonate as a standard.
[b]Chemical structures and numbers refer to Figure 3.
[c]PD indicates products; PD1, product 1; PD2, product 2; and so forth.
[d]For the glucose unit attached to salicyl alcohol.
[e]For the second glucose unit of the disaccharide attached to salicyl alcohol.
[f]For the third glucose unit of the trisaccharide attached to salicyl alcohol.

(PC1, PC2, $PC4_1$, $PC4_2$, and PC5 from the CGTase reaction and PD1, PD2, PD3, PD4, PD5, and PD6 from the dextransucrase reaction) and (*b*) those that only contained carbohydrate and no salicyl alcohol (PC10, PC11, PC12, and PC13 from the CGTase reaction and PD9 from dextransucrase reaction) are also given in Table 3.

PC1, PC2, PD1, and PD2 each had m/z $[M+Na]^+ = 309$, indicating a single D-glucopyranosyl unit was attached to salicyl alcohol (Table 3). $PC4_1$, $PC4_2$, PC5, PD3, PD4, and PD5 had an m/z $[M+Na]^+ = 471$, indicating that two glucose units were attached to salicyl alcohol to give salicyl alcohol analogue isomers (Table 3). PD9, PC10, and PC11 each had m/z 365 and were disaccharides, such as maltose or isomaltose (Table 3). PC12 and PC13, each with m/z 527, were trisaccharides (Table 3).

3.5. Determination of the structures of the products by NMR

The structures of the products were determined by 1D ^{1}H, 1D ^{13}C, ^{1}H/^{1}H COSY, and ^{1}H/^{13}C HMQC NMR. The major product from the dextransucrase reaction (PD1) and the minor product from the CGTase reaction (PC1) were identical and their ^{1}H- and ^{13}C-chemical shifts are given in Tables 4–7. The major product from the CGTase reaction (PC2) and the minor product from the dextransucrase reaction (PD2) were identical and their ^{1}H- and ^{13}C-chemical shifts are given in Tables 4–7.

For PD1 and PC1 there is only one anomeric proton (H-1) and one anomeric carbon (C-1) for the bound glucose unit. The H-1 chemical shift was at 5.03 ppm, with a coupling constant of 3.4 Hz and the ^{13}C-chemical shift for C-1 was at 98.3 ppm (Tables 4–7). These results

Table 7. ^{13}C NMR chemical shifts[a] for salicyl alcohol and its analogues produced by the reaction of dextransucrase with sucrose and salicyl alcohol

	Position[b]	PD1[c]	PD2[c]	PD3[c]	PD4[c]	PD5[c]	PD6[c]
Salicyl alcohol (SA)	*C*-1	154.9	154.2	155.0	155.2	154.0	154.9
	C-2	124.0	129.7	124.1	124.1	129.7	124.1
	C-3	131.4	130.1	131.6	131.7	130.1	131.5
	C-4	121.0	123.3	120.9	120.8	123.4	121.0
	C-5	130.5	130.0	130.5	130.7	130.0	130.8
	C-6	116.2	115.4	115.3	116.3	115.5	116.2
	C-7	66.0	59.9	65.9	66.4	59.8	66.4
Glc(I)[d]	*C-1*	98.3	97.0	98.4	98.4	96.8	98.6
	C-2	71.8	71.8	70.4	71.7	71.6	71.8
	C-3	73.6	73.5	80.2	73.8	74.0	73.9
	C-4	69.9	69.8	70.2	69.6	70.0	70.0
	C-5	72.3	73.1	72.1	70.7	71.7	70.9
	C-6	60.7	60.8	60.4	65.7	65.9	65.9
Glc(II)[e]	*C*-1			99.5	98.3	98.2	95.9
	C-2			72.1	71.8	71.8	76.0
	C-3			73.2	73.4	73.6	71.8
	C-4			69.9	69.7	69.9	69.9
	C-5			72.1	72.1	72.3	72.1
	C-6			60.6	60.8	60.9	60.9
Glc(III)[f]	*C*-1						96.8
	C-2						71.8
	C-3						73.4
	C-4						69.9
	C-5						72.3
	C-6						60.9

[a] Chemical shifts were measured at 125 MHz in D_2O at 25 °C, using 1,4-dioxane as a standard.
[b] For positions and chemical structures refer to Figure 3.
[c] PD indicates product; PD1, product 1; PD2, product 2; and so forth.
[d] For the glucose unit attached to salicyl alcohol.
[e] For the second glucose unit of the disaccharide attached to salicyl alcohol.
[f] For the third glucose unit of the trisaccharide attached to salicyl alcohol.

indicate that one D-glucopyranose was attached to salicyl alcohol by an α-linkage.[21,22] When the ^{13}C NMR spectra of PD1 and PC1 was compared with that of salicyl alcohol, there was a significant change from 60.1 to 66.0 ppm for C-7 carbon of salicyl alcohol, with only very small chemical shifts for the other salicyl alcohol carbons (Tables 5 and 7). These results show that the D-glucopyranose was attached to the hydroxyl group on C-7 of salicyl alcohol by an α-linkage.[13,23] PD1 and PC1, thus, have D-glucopyranose attached to the primary alcohol hydroxyl group of salicyl alcohol to give 7-α-D-glucopyranosyl salicyl alcohol (see Fig. 3B for the structure).

For the HMQC-spectra for PC2 and PD2 only one anomeric proton (H-1) and carbon (C-1) peak appeared for the bound glucose unit and the ^{1}H-chemical shift was at 5.03 ppm, with a coupling constant of 3.4 Hz and the ^{13}C-chemical shift was at 97.0 ppm (Tables 4–7). These results indicate that one D-glucopyranose was attached to salicyl alcohol by an α-linkage, similar to PC1 and PD1.[21,22] Previous studies[24,25] showed that when carbohydrate is bound to the phenolic hydroxyl of salicyl alcohol, there was no significant deviation of the chemical shift for the C-1 of salicyl alcohol, but there was a significant downfield shift of 0.4 ppm for the proton on C-6 of salicyl alcohol and a significant downfield ^{13}C shift of 4.8 ppm for the C-2 of salicyl alcohol. In our experiments, there was no significant shift for C-1 of salicyl alcohol (Table 5), but there was a significant downfield shift of about 0.33 ppm for the proton at the C-6 of salicyl alcohol and a significant downfield ^{13}C shift of 3.0 ppm for the C-2 of salicyl alcohol (Tables 4–7). The NMR results for PC2 and PD2, thus, indicate that one D-glucopyranose unit is attached to the C-1-phenolic hydroxyl group of salicyl alcohol, giving 1-α-D-glucopyranosyl salicyl alcohol.

PD3 and PD4 had spectra that were nearly identical to the spectra of PC1 and PD1 (7-α-D-glucopyranosyl salicyl alcohol), but both of these compounds also had two glucose units instead of one. PD3 differed from PD1 in the C-3 position of the glucose unit attached to salicyl alcohol. The ^{13}C-chemical shift for C-3 changed from 73.6 to 80.2 ppm (Tables 5 and 7). The C-1 of the second glucose unit had a ^{13}C-chemical shift of 99.5 ppm and a ^{1}H-chemical shift of 5.31 ppm, with a coupling constant of 3.4 Hz (Tables 4–7), indicating that it was α-linked. Thus, the second glucose unit was α-(1 → 3) linked to the first glucose unit, giving 7-α-D-nigerosyl salicyl alcohol.

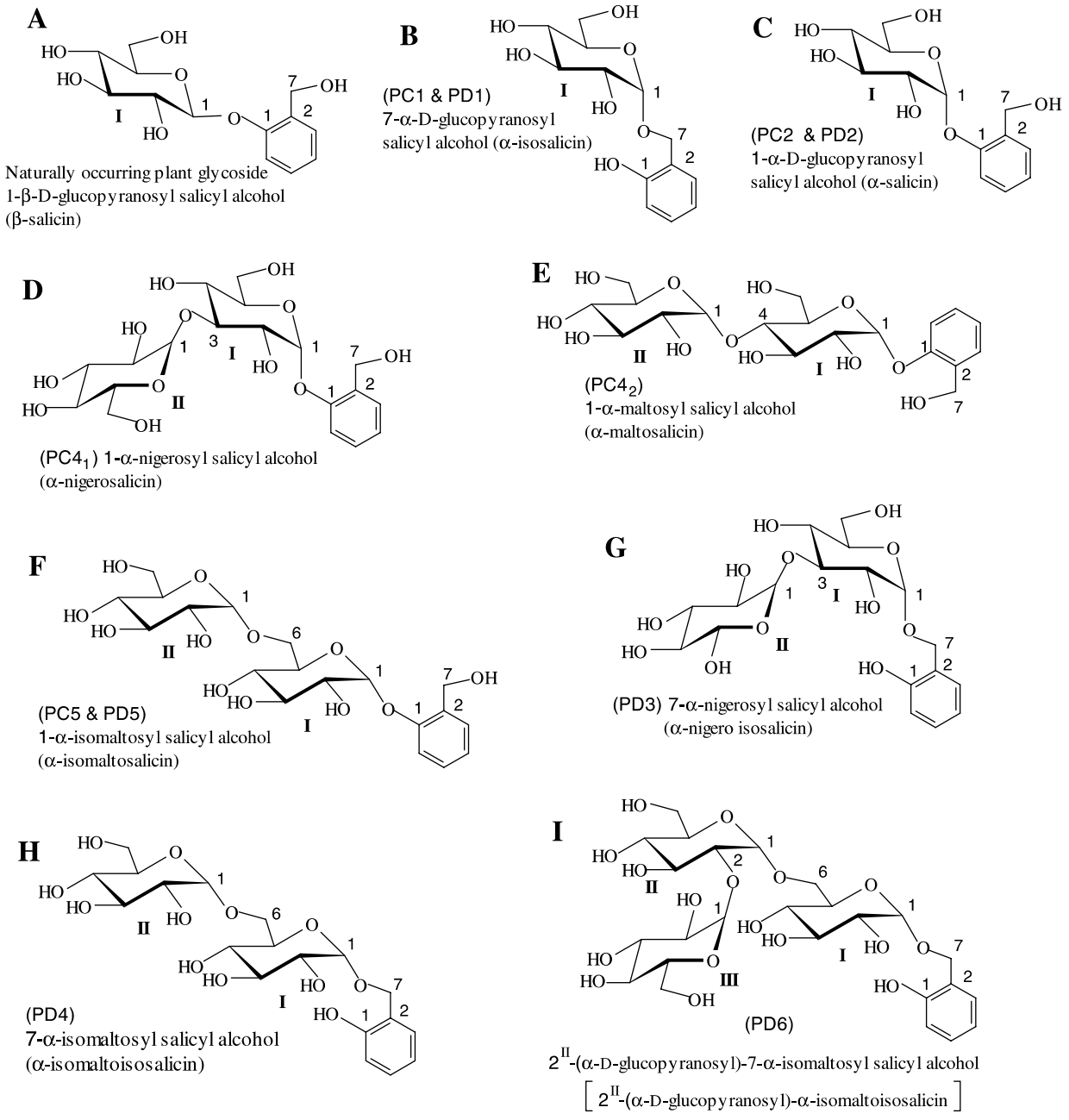

Figure 3. The chemical structures of β-salicin and the reaction products, α-salicin and α-isosalicin analogues, produced by the reactions of CGTase and B-742CB dextransucrase.

PD4 differed from PD3 by the C-6 carbon of the glucose unit attached to salicyl alcohol, which had a downfield shift of 5.0 ppm, from 60.7 to 65.7 ppm (Tables 4–7). PD4, thus, had the second glucose unit linked α-(1 → 6) to the first glucose unit, giving 7-α-isomaltosyl salicyl alcohol.

PD6 has three glucose units and its ^{1}H- and ^{13}C-chemical shifts were nearly identical to those of PD4, with the exception of the C-2 position of the second glucose unit in which there was a ^{13}C shift of 72.1–76.0 ppm, a downfield shift of 3.9 ppm. C-1 of the third glucose unit had ^{1}H-chemical shift of 5.09, with a coupling constant of 3.4 Hz (Tables 6 and 7), indicating that it was α-linked. The third glucose unit is, thus, linked α-(1 → 2) to the second glucose of the nigerosyl unit to give 2^{II}-(α-D-glucopyranosyl)-7-α-nigerosyl salicyl alcohol.

PC4$_1$, PC4$_2$, and PC5 are products produced by the CGTase reaction and PD5 is a product produced by the dextransucrase reaction. All four products have two glucose residues and the ^{1}H- and ^{13}C NMR spectra were very similar to the spectrum of PC2.

The spectrum of PC4$_1$ differed from PC2 by having a downfield ^{13}C shift from 73.5 to 79.6 ppm for the C-3 of the glucose attached to salicyl alcohol, and the C-1 of

the second glucose unit had a ^{13}C-chemical shift of 99.4 ppm and a ^{1}H-chemical shift of 5.45 ppm, with a coupling constant of 3.4 Hz (Tables 4 and 5). These results indicate that the second glucose unit is attached to the first glucose unit by an α-(1 → 3) linkage, giving 1-α-nigerosyl salicyl alcohol.

The spectrum for $PC4_2$ differed from PC2 by having a downfield ^{13}C shift from 69.8 to 77.1 ppm (Tables 4 and 5) for the C-4 of the first glucose unit attached to salicyl alcohol. The C-1 of the second glucose unit had a ^{13}C-chemical shift of 98.2 ppm and a ^{1}H-chemical shift of 5.42 ppm, with a coupling constant of 3.4 Hz (Tables 4 and 5), indicating that the second glucose unit was attached to the first glucose unit by an α-(1 → 4) linkage of PC2 to give, 1-α-maltosyl salicyl alcohol.

The spectra of PC5 and PD5 were identical and differed from PC2 by having a downfield ^{13}C shift from 60.8 to 65.9 ppm for the C-6 of the first glucose attached to salicyl alcohol (Tables 5 and 7). The C-1 of the second glucose unit had a ^{13}C-chemical shift of 98.2 ppm and ^{1}H-chemical shift of 4.85 ppm, with a coupling constant of 3.1 Hz (Tables 4–7). PC5 and PD5, thus, had a D-glucose unit attached α-(1 → 6) to the glucose unit of PC2 structure, to give 1-α-isomaltosyl salicyl alcohol (Tables 4–7).

3.6. NMR determination of the structures of carbohydrates (PC10–PC13, and PD9) not attached to salicyl alcohol

The carbohydrates, PC10–PC13, were produced by CGTase reaction and PD9 was produced by dextransucrase reaction during preparation of salicyl alcohol analogues. These carbohydrates products were not attached to salicyl alcohol. The ^{13}C-chemical shifts of PC10 and PC11 (Table 9) were identical to those previously reported for α,β-trehalose[26] and isomaltose,[27] respectively, and thus were identified as being α,β-trehalose and isomaltose.

The ^{13}C shifts of PC12 were almost identical to those of maltose,[26,28] with the exception of the C-3 of glucose (II) unit and a newly bound glucose (III) (Table 9). The newly bound glucose (III) unit has a ^{13}C-chemical shift of 99.5 ppm for C-1 and a ^{1}H-chemical shift of 5.33 ppm, with 3.4 Hz coupling constant (Tables 8 and 9). The ^{13}C-chemical shift of C-3 position of glucose (II) was changed from 73.8 ppm[26,28] to 79.7 ppm (Table 9). Thus, PC12 was identified as 3^{II}-α-D-glucopyranosyl maltose.

The ^{13}C shifts of P13 (Table 9) were very similar to that of the kojibiose,[26,28] with the exception of the C-4-position of the reducing end glucose unit and a newly bound glucose (II) (Table 9). The newly bound glucose (II) unit had a C-1 ^{13}C-chemical shift of 99.5 ppm and a ^{1}H-chemical shift of 5.44 ppm, with a coupling constant of 3.0 Hz (Tables 8 and 9). The ^{13}C-chemical shifts of the C-4 position of the first glucose unit was changed from 70.3 ppm[26,28] to 76.2 ppm (Table 9) and from 70.1 ppm[26,28] to 76.1 ppm (Table 9), respectively. P13 was, thus, identified as 4^{I}-α-D-glucopyranosyl kojibiose or 2^{I}-α-D-glucopyranosyl maltose.

The ^{1}H- (Table 8) and ^{13}C NMR results (Table 9) of PD9 were identical to those of previously reported α-isomaltulose or palatinose.[29,30] Therefore, PD9 was identified as 6-α-D-glucopyranosyl-α-D-fructofuranose or α-isomaltulose.

4. Discussion

Two kinds of reactions were used to synthesize salicin analogues. In the first synthesis, *B. macerans* CGTase catalyzed a transglycosylation reaction between cyclomaltohexaose and salicyl alcohol to give two products, 1-α-maltohexaosyl salicyl alcohol (major product) and 7-α-maltohexaosyl salicyl alcohol (minor product). The enzyme also catalyzed transglycosylation/disproportionation reactions with the maltohexaosyl unit attached to salicyl alcohol. To reduce the number of salicyl products and give a single D-glucose unit attached to salicyl alcohol, the reaction mixture was reacted with alpha amylase and glucoamylase, and then the free sugars were removed by fermentation with immobilized *S. cerevisiae*. The major product has a structure analogous to natural salicin, but with the D-glucopyranosyl moiety α-linked to the phenolic hydroxyl group of salicyl alcohol. The minor product has the D-glucopyranosyl moiety α-linked to the primary alcohol hydroxyl group of salicyl alcohol. Naturally occurring salicin has the D-glucopyranosyl β-linked to the phenolic group of salicyl alcohol. We have given common names to the newly synthesized analogues: α-salicin for the major analogue and α-isosalicin for the minor analogue, and β-salicin for the naturally occurring salicin (see Fig. 3A–C for the structures).

CGTase also synthesized three additional minor products. Their structures were determined to be: 1-α-nigerosyl salicyl alcohol, named α-nigerosalicin (Fig. 3D); 1-α-maltosyl salicyl alcohol, named α-maltosalicin (Fig. 3E); and 1-α-isomaltosyl salicyl alcohol, named α-isomaltosalicin (Fig. 3F).

In the first reaction with CGTase, before reaction with alpha amylase and glucoamylase, three products, resulting from the disproportionation reactions (PC3, PC6, and PC8) are salicin analogues with maltose, maltotriose, and maltotetraose attached to C-7 position of salicyl alcohol. PPA and GA hydrolyzed the carbohydrates on these analogues, leaving α-isosalicin.

The formation of nigerose and isomaltose attached to C-1 of salicyl alcohol to give α-nigerosalicin and α-isomaltosalicin were most probably formed by condensation reactions of D-glucose with α-salicin, catalyzed by glucoamylase.[31,32] Likewise, the formation of

Table 8. [1]H NMR chemical shifts[a] for carbohydrates produced from CGTase and dextransucrase reactions

Position	PC10[b]	PC11[b]	PC12[b]	PC13[b]	PD9[b]
	β-D-Glc(I)	α-D-Glc(I)	α-D-Glc(I)	α-D-Glc(I)	α-D-Glc(I)
H-1	4.63	5.21	5.20	5.43	4.95
	(7.9)	(3.4)	(3.4)	(3.1)	(<3.0)
H-2	3.39	3.50	3.56	3.65	3.55
H-3	3.51	3.69	3.96	4.09	3.73
H-4	3.41	3.39	3.64	3.69	3.39
H-5	3.45	3.70	3.92	3.95	3.69
H-6	3.69	3.73	3.76	3.79	3.75
H-6′	3.85	3.93	3.83		3.85
	α-D-Glc(II)	β-D-Glc(I)	β-D-Glc(I)	β-D-Glc(I)	β-D-Fru(II)
H-1	5.22	4.65	4.63	4.79	3.51
H-1′	(3.4)	(7.9)	(7.9)	(7.9)	3.63
H-2	3.57	3.22	3.25	3.41	—
H-3	3.73	3.44	3.75	3.86	4.10
H-4	3.43	3.39	3.64	3.68	4.18
H-5	3.92	3.70	3.84	3.58	3.95
H-6	3.74	3.73	3.76	3.75	3.68
H-6′	3.81	3.93	3.92	3.89	3.86
		D-Glc(II)	D-Glc(II)	D-Glc(II)	
H-1		4.92	5.38	5.44	
		(3.1)	(3.4)	(<3.0)	
H-2		3.52	3.66	3.54	
H-3		3.69	3.83	3.65	
H-4		3.39	3.64	3.40	
H-5		3.66	3.72	3.69	
H-6		3.73	3.75	3.77	
H-6′		3.81	3.82	3.86	
			D-Glc(III)	D-Glc(III)-α(I)[c]	
H-1			5.33	5.09	
			(3.4)	(3.1)	
H-2			3.54	3.53	
H-3			3.72	3.77	
H-4			3.40	3.43	
H-5			3.99	3.89	
H-6			3.77	3.73	
H-6′			3.89	3.83	
				D-Glc(III)-β(I)[c]	
H-1				5.39	
				(3.1)	
H-2				3.52	
H-3				3.73	
H-4				3.42	
H-5				3.98	
H-6				3.78	
H-6′				3.85	

[a] Chemical shifts and coupling constants (in parentheses, Hz) were measured at 500 MHz in D_2O at 25 °C, using sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) as a standard.

[b] PC = products from CGTase reaction and PD = product from dextransucrase reaction.

[c] α-D-Glucose unit connected to α- and β-anomer of reducing-end glucose, respectively.

α,β-trehalose, isomaltose, 3^{II}-α-D-glucopyranosyl maltose, and 2^{I}-α-D-glucopyranosyl maltose saccharides were also most probably the results of glucoamylase reversion reactions that give condensed saccharide

Table 9. ^{13}C NMR chemical shifts[a] for the carbohydrates produced from the reactions of CGTase and dextransucrase

Position	PC10[b]	PC11[b]	PC12[b]	PC13[b]	PD9[b]
	β-D-Glc(I)	α-D-Glc(I)	α-D-Glc(I)	α-D-Glc(I)	α-D-Glc(I)
C-1	103.3	92.6	92.3	89.5	98.6
C-2	73.5	71.8	75.0	76.0	71.8
C-3	75.8	73.4	73.5	71.8	73.5
C-4	69.7	69.9	77.2	76.2	69.9
C-5	76.5	70.4	70.3	70.1	72.3
C-6	61.0	66.0	60.9	60.8	61.0
	α-D-Glc(II)	β-D-Glc(I)	β-D-Glc(I)	β-D-Glc(I)	β-D-Fru(II)
C-1	100.6	96.4	96.2	96.5	63.0
C-2	71.8	74.7	74.4	78.8	102.2
C-3	73.2	76.3	76.5	75.3	75.7
C-4	69.7	69.8	77.2	76.1	74.9
C-5	73.1	74.4	75.1	74.7	79.4
C-6	60.8	66.0	61.1	61.1	68.3
		D-Glc(II)	D-Glc(II)	D-Glc(II)	
C-1		98.3	100.4	99.3/99.6	
C-2		72.2	70.6	72.1	
C-3		73.4	79.7	73.0	
C-4		69.9	70.1	69.6	
C-5		71.8	72.9	73.0	
C-6		60.8	60.9	60.8	
			D-Glc(III)	D-Glc(III)-α(I)[c]	
C-1			99.5	96.5	
C-2			72.1	72.1	
C-3			73.2	73.2	
C-4			69.8	69.6	
C-5			72.2	71.9	
C-6			60.7	60.6	
				D-Glc(III)-β(I)[c]	
C-1				98.0	
C-2				72.2	
C-3				73.1	
C-4				69.6	
C-5				71.8	
C-6				60.6	

[a] Chemical shifts were measured at 125 MHz in D_2O at 25 °C, using 1,4-dioxane as a standard.
[b] PC refers to products produced by CGTase and PD refers to the product produced by dextransucrase.
[c] α-D-Glucose unit connected to α- and β-anomer of reducing-end glucose, respectively.

products from the relatively high amounts of D-glucose that is formed by the action of glucoamylase on the maltodextrin chains attached to salicyl alcohol and the maltodextrins produced by the CGTase disproportionation reactions.

In the second synthesis, *L. mesenteroides* B-742CB dextransucrase catalyzed transglycosylation reactions in which D-glucopyranose is transferred from sucrose to the primary alcohol hydroxyl group to give α-isosalicin, as the major product, in contrast to CGTase, which gave α-salicin as the major product. This glucose unit also acts as an acceptor and a sequential series of glucose units are transferred to give isomaltodextrinyl chains attached to salicyl alcohol. These chains were reduced to a single glucose unit attached to the primary alcohol hydroxyl of salicyl alcohol by the action of dextranase and glucoamylase, which gave α-isosalicin as the major product (see Fig. 3B).

B-742 Dextransucrase also formed three additional minor α-isosalicin analogues by the acceptor reaction transfer of D-glucopyranose from sucrose to the D-glucopyranosyl moiety of α-isosalicin to form α-(1 → 3) and α-(1 → 6) glycosidic linkages to give α-nigeroisosalicin (Fig. 3G) and α-isomaltoisosalicin (Fig. 3H),

respectively, and the transfer of D-glucopyranose from sucrose to the nonreducing end of the isomaltose unit of α-isomaltoisosalicin to form an α-(1 → 2) glycosidic linkage to give 2^{II}-(α-D-glucopyranosyl)-α-isomaltoisosalicin (Fig. 3I). In addition to these α-isosalicin analogues, B-742CB dextransucrase also formed minor amounts of α-salicin and transferred D-glucopyranose from sucrose to form an α-(1 → 6) linkage to the D-glucopyranosyl moiety of α-salicin to give 1-α-isomaltosyl salicyl alcohol or α-isomaltosalicin (Fig. 3F).

It is known that the major active metabolite from β-salicin in serum is salicylic acid.[3,7] β-Salicin is absorbed only to a small extent in the small intestine after oral administration.[3] It is hydrolyzed to salicyl alcohol, which is readily absorbed from the small intestine by the action of β-glycosidases secreted by the intestinal microflora[3,33–35] or by β-galactosidase in the mucous membrane of the small intestine.[35] The absorbed salicyl alcohol is rapidly oxidized to salicylic acid in the human organs (liver, kidney, and lung) of the body.[36,37] Consequently, when β-salicin is taken orally, it is poorly absorbed, but it is slowly transformed into salicyl alcohol by intestinal bacteria, and the absorbed salicyl alcohol is oxidized to the active metabolite, salicylic acid.

Although it has not yet been demonstrated, it might be expected that α-salicin and its analogues, α-isosalicin, α-nigerosalicin, α-maltosalicin, and α-isomaltosalicin that have been synthesized in this study, would be readily hydrolyzed by the α-glucosidases secreted by the brush border cells that line the small intestinal wall.[38] This would then facilitate the absorption of salicyl alcohol and would make it a mild but effective antipyretic and analgesic prodrug with none of the gastric injury and side effects that have been ascribed to aspirin (sodium acetylsalicylate).

In conclusion, two types of salicin prodrugs have been enzymatically synthesized. The reaction of *B. macerans* CGTase with cyclomaltohexaose and salicyl alcohol gave α-salicin as a major product and the reaction of *L. mesenteroides* B-742CB dextransucrase with sucrose and salicyl alcohol gave α-isosalicin as the major product.

References

1. Robyt, J. F. *Essentials of Carbohydrate Chemistry*; Springer: New York, 1998; pp 64–68.
2. Křen, V. Chemical Biology and Biomedicine of Glycosylated Natural Compounds. In *Glycoscience: Chemistry and Chemical Biology*; Fraser-Reid, B., Tatsuta, K., Thiem, J., Eds.; Springer: Berlin, 2001; Vol. III, pp 2471–2529.
3. Akao, T.; Yoshino, T.; Kobashi, K.; Hattori, M. *Planta Med.* **2002**, *68*, 714–718.
4. Matito, C.; Mastorakou, F.; Centelles, J. J.; Torres, J. L.; Cascante, M. *Eur. J. Nutr.* **2003**, *42*, 43–49.
5. Arakawa, H.; Funayama, M.; Yamamoto, R.; Nishino, T. *Jpn. Kokai Tokkyo Koho* 06284897, **1994**.
6. Shimoide, A.; Watanabe, M.; Donho, M. *Jpn. Kokai Tokkyo Koho* 08173183, **1996**.
7. Okano, Y.; Masaki, H.; Ogawa, A. *Jpn. Kokai Tokkyo Koho* 11116426, **1999**.
8. Schmid, B.; Kötter, I.; Heide, L. *Eur. J. Pharmacol.* **2001**, *57*, 387–391.
9. French, D. *Adv. Carbohydr. Chem.* **1957**, *12*, 225–231.
10. Robyt, J. F. *Adv. Carbohydr. Chem. Biochem.* **1995**, *51*, 151–162.
11. Kobayashi, S.; Kainuma, K.; Suzuki, S. *Carbohydr. Res.* **1978**, *61*, 229–238.
12. Lee, S.-B.; Robyt, J. F. *Carbohydr. Res.* **2001**, *336*, 47–53.
13. Yoon, S.-H.; Robyt, J. F. *Carbohydr. Res.* **2002**, *337*, 2427–2435.
14. Kitaoka, M.; Robyt, J. F. *Enzyme Microb. Technol.* **1998**, *23*, 386–391.
15. Yoon, S.-H.; Mukerjea, R.; Robyt, J. F. *Carbohydr. Res.* **2003**, *338*, 1127–1132.
16. Robyt, J. F. Thin-layer Chromatography of Carbohydrates. In *Encyclopedia of Separation Science*; Wilson, I. D., Cooke, M., Poole, C. F., Eds.; Academic: New York, 2000; Vol. 5, pp 2235–2244.
17. Robyt, J. F.; Mukerjea, R. *Carbohydr. Res.* **1994**, *251*, 187–202.
18. Fox, J. D.; Robyt, J. F. *Anal. Biochem.* **1991**, *195*, 93–96.
19. Robyt, J. F.; White, B. J. *Biochemical Techniques—Theory and Practice*; Waveland: Prospect Heights, IL, 1990; pp 82–86 and 106–111.
20. Kazmaier, T.; Toth, S.; Zapp, J.; Harding, M.; Kuhn, R. *Fresenius J. Anal. Chem.* **1998**, *361*, 473–478.
21. Bock, K.; Pedersen, C. H. *J. Carbohydr. Chem.* **1984**, *3*, 581–592.
22. Duus, J. Ø.; Gotfredsen, C. H.; Bock, K. *Chem. Rev.* **2000**, *100*, 4589–4614.
23. Yoon, S.-H.; Robyt, J. F. *Carbohydr. Res.* **2002**, *337*, 509–516.
24. Tamaki, A.; Ide, T.; Otsuka, H. J. *J. Nat. Prod.* **2000**, *63*, 1417–1419.
25. Itoh, A.; Tanahashi, T.; Inoue, K.; Kuwajima, H.; Wu, H.-X. *Chem. Pharm. Bull.* **2001**, *49*, 1343–1345.
26. Bock, K.; Thøgersen, H. *Annu. Rep. NMR Spectro.* **1982**, *13*, 1–57.
27. Vetere, A.; Gamini, A.; Campa, C.; Paoletti, S. *Biochem. Biophys. Res. Commun.* **2000**, *274*, 99–104.
28. Hoffman, R. E.; Christofides, J. C.; Davies, D. B.; Lawson, C. J. *Carbohyr. Res.* **1986**, *153*, 1–16.
29. Jarrell, H. C.; Conway, T. F.; Moyna, P.; Smith, I. C. P. *Carbohydr. Res.* **1979**, *76*, 45–57.
30. Thompson, J.; Robrish, S. A.; Pikis, A.; Brust, A.; Lichtenthaler, F. W. *Carbohydr. Res.* **2001**, *331*, 149–161.
31. Underkofler, L. A.; Denault, L. J.; Hou, E. F. *Stärke* **1965**, *17*, 179–184.
32. Zivko, L. N.; Meagher, M. M.; Reilly, P. J. *Biotechnol. Bioeng.* **1989**, *34*, 694–704.
33. Mallett, A. K.; Bearne, C. A.; Rowland, I. R.; Farthing, M. J. G.; Cole, C. B.; Fuller, R. J. *Appl. Bacteriol.* **1987**, *63*, 39–45.
34. Underkofler, L. A.; Denault, L. J.; Hou, E. F. *Stärke* **1965**, *17*, 179–184.
35. Zivko, L. N.; Meagher, M. M.; Reilly, P. J. *Biotechnol. Bioeng.* **1989**, *34*, 694–704.
36. Fotsch, G.; Pfeifer, S. *Pharmazie* **1989**, *44*, 710–712.
37. Steinegger, E.; Hövel, H. *Pharm. Acta Helv.* **1972**, *47*, 222–234.
38. Gray, G. M.; Lally, B. C.; Conklin, K. A. *J. Biol. Chem.* **1978**, *254*, 6038–6042.