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A survey of the nature of glucose acylation reactions in plant extracts

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Abstract

A variety of plant species have been shown to catalyse anomeric acyl exchange from a number of 1-*O*-fatty-acyl-β-glucoses as donor substrates to [^{14}C]-glucose as an acceptor. The activity in wild tomato *Lycopersicon pennellii* has been analysed in detail by using analogs of glucose as acceptors and a number of 1-*O*-acyl-β-glucoses and 1-*O*-acyl-2-deoxyglucoses as acyl donors. Compared to 1-*O*-isobutyryl-β-glucose, the analogous 1-*O*-isobutyryl-β-2-deoxyglucose is an effective donor both to glucose (ca. 75%) and to 2-deoxyglucose (ca. 95%). On the contrary, compared to 1-*O*-isobutyryl-β-glucose, 1-*O*-isobutyryl-α-2-deoxyglucose is a poor donor both to glucose (ca. 4%) and to deoxyglucose (ca. 6%). The glucose analogs free at the anomeric center such as 3-*O*-methylglucose and 2-deoxyglucose are competent acyl acceptors from various 1-*O*-acyl-β-glucoses whereas 1-*O*-methylglucose is not. The primary initial product of acyl transfer from 1-*O*-isobutyryl-β-glucose to [^{14}C]-glucose is β-glucosidase-cleavable 1-*O*-acyl-[^{14}C]-β-glucose, whereas the isobutyryl-[^{14}C]-3-*O*-methylglucose generated from 1-*O*-isobutyryl-β-glucose and [^{14}C]-3-*O*-methylglucose is β-glucosidase-resistant. The transfer of the acyl group to 3-*O*-methylglucose occurs at the anomeric center; therefore the resistance to β-glucosidase reflects the strict specificity of β-glucosidase for glucose.

Keywords: *Lycopersicon pennellii*; Solanaceae; Acyl exchange; Triacylglucose biosynthesis; Acylglucose; Acylsugar; Isobutyroyl-glucose

1. Introduction

Because of their surfactant nature, fatty acid esters of glucose and sucrose are widely used in food and cosmetic industries as emulsifiers and emollients. Recently, Olestra, a sucrose polyester, was approved by the US Food and Drug Administration as a cooking-oil alternative. So far the production of these esters has depended on partial or purely chemical processes. Interestingly, similar esters of glucose and sucrose (acylsugars) are produced by a variety of Solanaceous plant species including wild tomato, *Lycopersicon pennellii* (Burke, Goldsby & Mudd, 1987; King, Calhoun, Singh & Boucher, 1993). The acylsugars of the wild tomato are the subject of continuous studies primarily because of their insect-deterring properties (Goffreda, Steffens & Mutschler, 1990; Hawthorne, Shapiro, Tingey & Mutschler, 1992; Juvik, Shapiro, Young & Mutschler, 1994; Liedl et al., 1995; Rodriguez, Tingey & Mutschler, 1993). Some of these recent studies have focused on the biosynthesis of these acylsugars (Ghangas & Steffens, 1993, 1995; Kuai, Ghangas & Steffens, 1997; Li, 1998). Such studies are likely to lead to economical and/or environment-friendly methods for the production of acylsugars.

The wild tomato, *L. pennellii* LA716, contains a unique fatty acid activation and transacylation mechanism implicated in the biosynthesis of its 2,3,4-triacylglucoses (Ghangas & Steffens, 1993, 1995). The activation reaction generates high-energy monoacylglucoses (1-*O*-acyl-β-glucoses) from UDPGlc and free fatty acids. The energetics for the formation of similar 1-*O*-acyl-β-glucoses and the standard free energy of

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Table 1
A survey for the presence of anomeric acyl exchange activities in plants: transfer of isobutyrate and laurate from 1-*O*-isobutyryl-β-D-glucose and 1-*O*-lauroyl-β-D-glucose, respectively, to [^{14}C]-glucose by *L. pennellii* and other species[a]

Plant	pmol acyl groups incorporated/mg protein leading to	
	monoisobutyryl-[^{14}C]-Glc	monolauroyl-[^{14}C]-Glc
Lycopersicon pennellii	976	1088
Lycopersicon esculentum	150	26
Solanum berthaultii	747	374
Nicotiana tabacum	467	282
Arabidopsis thaliana	330	115
Triticum aesticum	42	16
Phaseolus vulgaris	611	282
Cucumis melo	115	23

[a] Reactions were carried out for 1 h as described in the text using 40 μM [^{14}C]-glucose (300 mCi/mmol), 1.25 mg/mL leaf protein, 2 mM donor in 50 mM NaPi pH 6.5 at 37°C. The products were separated and monitored by TLC (Ghangas & Steffens, 1993).

their hydrolysis have recently been estimated (Mock & Strack, 1993). Two soluble proteins from *L. pennellii*, responsible for the activation reactions have been partially purified (Kuai et al., 1997). An acyltransferase enzyme has also been purified whose sequence is homologous to serine-type carboxypeptidases and contains a catalytic triad (Asp–His–Ser) in the active site, similar to those found in a variety of serine proteases (Li, 1998). This acyltransferase catalyzes acyl exchange i.e. the transfer of anomeric acyl moiety of the 1-*O*-acyl-β-glucoses to the anomeric position of β-glucose (Ghangas & Steffens, 1995). The enzyme is inhibited by diisopropylfluorophosphate, an inhibitor of serine proteases. Presumably, the acyltransferase forms an Enz–Ser–acyl intermediate from which the acyl group is transferred to a glucose molecule near the active site of the enzyme. The same acyltransferase catalyzes the transfer of the acyl group from a molecule of 1-*O*-acyl-β-glucose to another 1-*O*-acyl-β-glucose molecule resulting in the formation of a diacylglucose and release of a glucose. This reaction is termed disproportionation (Ghangas & Steffens, 1995). While the activation and disproportionation reactions appear crucial to the synthesis of polyacylated glucoses, the role of acyl exchange in the synthesis of polyacylated glucoses is not clear. In vitro, nevertheless, acyl exchange can be used to prepare radiolabeled 1-*O*-acyl-β-glucoses (Ghangas & Steffens, 1995; Denzel, Weisemann & Gross, 1988).

In this communication the results of a study on acyl exchange in various plants are reported, together with the first chemical synthesis of α- and β-1-*O*-isobutyryl-2-deoxyglucoses. Using these and other analogs, the donor and acceptor substrates for acyl exchange and disproportionation in *L. pennellii* (LA716) were analysed. During this work features of β-glucosidase specificity were uncovered which might be useful in preserving the group transfer potential of 1-*O*-acyl linkages generated from UDPGlc and free fatty acids.

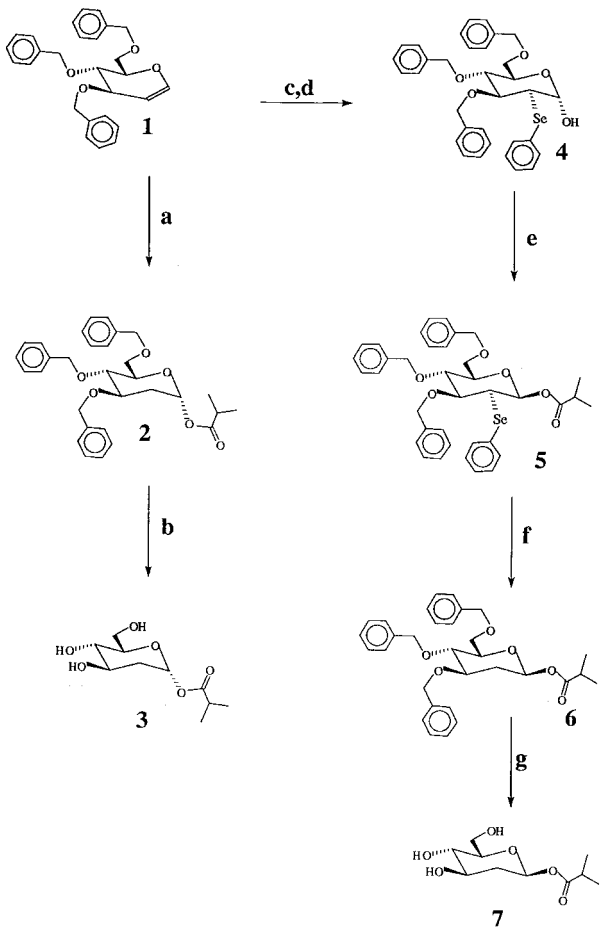

Fig. 1. Stereocontrolled syntheses of α- and β-1-*O*-isobutyryl-2-deoxyglucoses. (**1**) 3,4,6-Tri-*O*-benzyl-D-glucal. (**2**) 1-*O*-isobutyryl-2-deoxy-3,4,6-tri-*O*-benzyl-α-glucopyranose. (**3**) 1-*O*-isobutyryl-2-deoxy-α-glucopyranose. (**4**) 2-deoxy-2-selenophenyl-3,4,6-tri-*O*-benzyl-α-glucopyranose. (**5**) 1-*O*-isobutyryl-2-deoxy-2-selenophenyl-3,4,6-tri-*O*-benzyl-β-glucopyranose. (**6**) 1-*O*-isobutyryl-2-deoxy-3,4,6-tri-*O*-benzyl-β-glucopyranose. (**7**) 1-*O*-isobutyryl-2-deoxy-β-glucopyranose. (**a**) $Ph_3P{\cdot}HBr$, isobutyric acid. (**b**) $Pd/C/H_2$, EtOAc. (**c**) PhSeCl. (**d**) Na_2CO_3, H_2O. (**e**) Ph_3P, isobutyric acid, DEAD. (**f**) ACHN, Bu_3Sn reduction. (**g**) $Pd/C/H_2$, EtOAc.

Table 2
Synthesis of monoacyl glucoses and monoacyl-2-deoxyglucoses from acyl donor analogs and ^{14}C-labeled sugar-acceptors[a]

Donor	% of [^{14}C]- monoacyl product generated from	
	[^{14}C]-Glc	[^{4}C]-2-d-Glc
1-*O*-Isobutyryl-β-Glc	8.19	3.0
1-*O*-Isobutyryl-2-deoxy-β-Glc	6.20	2.83
1-*O*-Isobutyryl-2-deoxy-α-Glc	0.31	0.18

[a] Reactions were carried out as described in the text using 50 μM [^{14}C]-glucose (or [^{14}C]-2-deoxy-glucose), 2.5 mg/mL *L. pennellii* protein, 2.5 mM donors in 50 mM NaPi pH 6.5, 37°C. The products were separated and monitored by TLC (Ghangas & Steffens, 1993).

2. Results and discussion

2.1. A survey of acyl exchange in various plants

Our previous work shows that acyl exchange is present in wild tomato *L. pennellii* (LA716) that produces acylglucoses. Measurable levels of acyl exchange were also found in *L. esculentum*, a plant that does not produce such acylsugars (Ghangas & Steffens, 1995). The new data show that acyl exchange from 1-*O*-isobutyryl-β-glucose (also termed 1-*O*-isobutyroyl-β-glucose) to glucose can be demonstrated in a wide variety of plants (Table 1). Catalytic acyl transfer occurs also from 1-*O*-lauroyl-β-glucose, although the ratios of isobutyryl and lauroyl transfer activities are not proportional across the species investigated. The differences in acyl transfer from 1-*O*-isobutyryl-β-glucose vs. 1-*O*-lauroyl-β-glucose in various plants could result from a single enzyme that shows chain-length differences or from isozymes that prefer substrates with different chain length. However, other protein and nonprotein factors could also modulate these activities. It was also observed that activation activity responsible for the generation of 1-*O*-acyl-β-glucoses from UDPGlc and free fatty acids is found in a wide variety of plants (unpublished). These findings suggest multiple biological roles in plants for these activities.

2.2. Chemical syntheses of 1-O-isobutyryl-2-deoxy-α/β-glucopyranoses

In order to further probe the specificity of acyl exchange, the 1-*O*-isobutyryl-2-deoxy-α/β-glucoses were synthesized from 3,4,6-tri-*O*-benzyl-D-glucal (Fig. 1). The synthetic strategy was designed to obtain the fully benzylated α- and β-precursors **2** and **6** of the final products **3** and **7** respectively (Fig. 1). Such precursors (**2** and **6**) are stable and yield the final products upon hydrogenolysis.

The synthesis of the α-precursor was carried out by reacting 3,4,6-tri-*O*-benzyl-D-glucal **1** with isobutyric acid in the presence of catalytic amounts of triphenylphosphine hydrobromide ($Ph_3P{\cdot}HBr$) (Bolitt, Mioskowski, Lee & Falck, 1990). Silica gel purification gave the pure α- precursor **2**. Catalytic hydrogenolysis resulted in pure 1-*O*-isobutyryl-2-deoxy-α-glucopyranose **3** with an overall yield of 40%.

Starting again with 3,4,6-tri-*O*-benzyl-D-glucal **1**, the synthesis of the β- precursor **6** and the final product **7** are also shown in Fig. 1. In a one-pot procedure the protected D-glucal was first reacted with phenylselenenyl chloride followed by treatment with aqueous Na_2CO_3. The reaction with phenylselenenyl chloride generates a mixture of unstable anomeric chlorides. Hydrolysis of the anomeric chlorides with aqueous Na_2CO_3 generates an equilibrium mixture from which the predominant α- isomer **4** was purified by flash chromatography. Esterification of α-OH of **4** with isobutyric acid using the Mitsunobu set of reagents (Mitsunobu, 1981) resulted in invertion of configuration at the anomeric carbon thus giving pure β-isomer **5**. Tributyltin hydride reduction of **5** cleanly removed the selenophenyl group and gave pure β-precursor **6**. Catalytic hydrogenolysis of **6** followed by flash purification gave **7** in an overall yield of 4% starting from **1**.

As compared to the α-isomer **2** the β-isomer **6** underwent slow hydrogenolysis. When hydrogenolysis was carried out in EtOH or MeOH instead of in EtOAc, loss of the isobutyryl group was observed.

2.3. Acyl exchange from 1-O-isobutyryl-2-deoxy-α/β-glucopyranoses

The results in Table 2 show that catalytic acyl transfer to free glucose and 2-deoxy-glucose occurs efficiently from 1-*O*-isobutyryl-β-glucose and 1-*O*-isobutyryl-β-2-deoxy-glucose. These results are consistent with the enzyme forming an active Enz–Ser–isobutyryl intermediate both from 1-*O*-isobutyryl-β-glucose and 1-*O*-isobutyryl-β-2-deoxy-glucose. It is interesting to note that acyl transfer from 1-*O*-isobutyryl-β-2-deoxy-glucose is more efficient to glucose than it is to 2-deoxy-glucose. Furthermore, the transfer from 1-*O*-isobutyryl-β-2-deoxy-glucose is about 20 fold faster than it is from 1-*O*-isobutyryl-α-2-deoxy-glucose.

The acyl exchange from 1-*O*-isobutyryl-β-2-deoxy-glucose to glucose has a novel synthetic utility as fol-

Table 3
Synthesis of mono-, di- and triacyl glucoses from various acyl donors and ^{14}C-labeled sugar-acceptors by *L. pennellii* leaf extracts[a]

Donor	[^{14}C]-acceptor	% of [^{14}C]-acceptor incorporated into		
		monoacyl	diacyl	triacyl
1-*O*-Isobutyryl-β-Glc	Glc	13.58	0.19	0.0
1-*O*-Lauroyl-β-Glc	Glc	0.59	0.35	0.08
1-*O*-Palmitoyl-β-Glc	Glc	0.26	0.19	0.0
1-*O*-Isobutyryl-β-Glc	2-d-Glc	6.52	0.0	0.0
1-*O*-Lauroyl-β-Glc	2-d-Glc	1.42	0.35	0.0
1-*O*-Palmitoyl-β-Glc	2-d-Glc	0.61	0.34	0.0
1-*O*-Isobutyryl-β-Glc	3-*O*-Me-Glc	11.17	0.15	0.0
1-*O*-Lauroyl-β-Glc	3-*O*-Me-Glc	0.91	0.19	0.0
1-*O*-Palmitoyl-β-Glc	3-*O*-Me-Glc	0.19	0.13	0.0

[a] Reactions were carried out for 6 h as described in the text using 50 μM [^{14}C]-glucose, 2.5 mg/mL protein, 2.5 mM donors in 50 mM NaPi pH 6.5, 37°C. The products were separated and monitored by TLC (Ghangas & Steffens, 1993).

lows. Since 1-*O*-isobutyryl-β-2-deoxy-glucose moves faster on silica gel than 1-*O*-isobutyryl-β-glucose, the availability of 1-*O*-isobutyryl-β-2-deoxy-glucose provides a new method for synthesizing 1-*O*-isobutyryl-[^{14}C]-β-glucose from [^{14}C]-glucose and 1-*O*-isobutyryl-β-2-deoxy-glucose. The chemical synthesis of 1-*O*-isobutyryl-[^{14}C]-β-glucose of known and high specific activity is expensive and time-consuming.

2.4. Acyl transfer from various 1-O-acyl-β-glucoses to sugar acceptors

Isobutyryl-, lauroyl- and palmitoyl-groups are transferred from their respective 1-*O*-acyl-β-glucoses not only to glucose but also to 2-deoxy-glucose and 3-*O*-methyl-glucose (Table 3). This monoacylation is followed by the formation of diacyl-glucose(s) and diacyl-3-*O*-methyl-glucose(s), whereas the formation of diisobutyryl-2-deoxy-glucose(s) is characteristically absent in such reactions. These results are consistent with the NMR spectral data that show 1,2-diisobutyryl glucose as a major product of 1-*O*-isobutyryl-β-glucose disproportionation by the enzyme found in *L. Pennellii* (not shown). Furthermore, on silica gel TLC plates the synthetic 1,2-diisobutyryl-β-glucose comigrates with an enzyme-generated diisobutyryl glucose from 1-*O*-isobutyryl-β-glucose. Low concentrations (up to 1 mM) of other sugars do not compete with anomeric transfer to glucose from 1-*O*-isobutyryl-β-D-glucose. Such competition becomes apparent at 200 fold molar excess of some other sugars, provided the anomeric center is available (Table 4). Note that 1-*O*-methyl-glucose does not compete even at this high level. This is consistent with the observation that 1-*O*-methyl-[U-^{14}C]glucose is not an acceptor substrate in acyl exchange assays (data not shown).

Table 4
Inhibition of monoisobutyryl-[^{14}C]-glucose biosynthesis generated through catalytic acyl exchange from 1-*O*-isobutyryl-β-D-glucose and [^{14}C]-glucose by the *L. pennellii* protein extract[a]

Competitor	% cpm in monoacylglucose[a]
None	100
+10 mM glucose	14
+10 mM 2-deoxy-glucose	37
+10 mM 3-*O*-methyl-glucose	43
+10 mM 1-thio-glucose	62
+10 mM 1-*O*-methyl-β-D-glucose	98
+10 mM galactose	43

[a] Reactions were carried out for 1 h as described in the text using 50 μM [^{14}C]-glucose, 2.5 mg/mL protein, 2.5 mM donor in 50 mM NaPi pH 6.5, 37°C. The products were separated and monitored by TLC (Ghangas & Steffens, 1993).

The formation of [^{14}C]-diacylglucoses from 1-*O*-isobutyryl-β-glucose and [^{14}C]-glucose (or [^{14}C]-3-*O*-methylglucose) is the result of disproportionations (Table 3). The absence of such diacylglucoses from 1-*O*-isobutyryl-β-glucose and [^{14}C]-2-deoxy-glucose suggests that the 2-OH group on glucose plays a major role in disproportionation and thus in the enzyme-catalyzed synthesis of higher-order acylated-glucoses. Disproportionation enzymes involved in the biosynthesis of other diacyl molecules have been identified in other plant systems (Dahlbender & Strack, 1984, 1986; Kojima & Kondo, 1985; Schmidt, Denzel, Schilling & Gross, 1987; Villegas, Shimokawa, Okuyama & Kojima, 1986). The activity from oak leaves is involved in the disproportionation of β-glucogallin (1-*O*-galloyl-β-glucose) for the biosynthesis of gallotannins (Schmidt et al., 1987). However, in that case the primary initial product is 1,6-di-galloyl-glucose and not 1,2-di-galloyl-glucose.

2.5. Susceptibility of acyl exchange products to β-glucosidase

The initial primary product of acyl transfer from 1-*O*-isobutyryl-β-glucose to [^{14}C]-glucose (Table 3) is susceptible to almond β-glucosidase. The acyl product generated from 3-*O*-methyl-glucose under the same conditions is completely resistant to β-glucosidase (not shown). Mass spectral analysis of this β-glucosidase-resistant product showed it to be 1-*O*-isobutyryl-3-*O*-methyl-glucose. The peaks at *m*/*z* 319 and *m*/*z* 71 confirmed the identity of the isobutyryl moiety. The origin of other peaks at *m*/*z* 303, *m*/*z* 242, *m*/*z* 200, *m*/*z* 169, *m*/*z* 140, *m*/*z* 115, *m*/*z* 98 were all characteristic of 3-*O*-methylglucopyranose structure (De Jongh & Bieman, 1963). The monoacyl-2-deoxy-glucose generated from [^{14}C]-2-deoxy-glucose and 1-*O*-isobutyryl-β-glucose is also susceptible to β-glucosidase although the rate is somewhat slower as compared with the product generated from [^{14}C]-glucose and 1-*O*-isobutyryl-β-glucose (data not shown).

The complete resistance of the 1-*O*-isobutyryl-3-*O*-methyl-glucose to almond β-glucosidase is consistent with previous observations that glucosidases usually require an unsubstituted glucosidic moiety for activity (Selmar, 1993; Esen, 1993). This would indicate that 1,*n*-diacyl-β-glucoses, such as generated through disproportionation of 1-*O*-isobutyryl-β-glucose, should be resistant to β-glucosidase action as well. Indeed, synthetic 1,*n*-diisobutyryl-β-glucoses (1-*O*-[1-^{14}C-isobutyryl],*n*-isobutyryl-β-glucoses) are completely resistant to β-glucosidase (data not shown).

2.6. Acyl exchange in vivo

Exogenous 1-*O*-isobutyryl-β-glucose is hydrolysed during uptake by *L. pennellii* leaves and thus cannot be used for donor studies in vivo. Therefore the nascent donor 1-*O*-isobutyryl-β-glucose generated from isobutyrate co-fed with the nonmetabolizable acceptor sugars 3-*O*-methyl-glucose or 2-deoxy-glucose was relied upon. In contrast to the results in vitro, no significant formation of monoisobutyryl 2-deoxy-glucose occurred in the wild tomato leaves fed with 2-deoxy-glucose and isobutyric acid; what appeared to be a weak formation of monoisobutyryl-3-O-methylglucose, however, could be observed in such in vivo experiments (not shown). Administration of [^{14}C]-glucose to leaves co-fed with isobutyrate was uninformative since the label was distributed in a random manner. The lack of appreciable in vivo transacylation from 1-*O*-isobutyryl-β-glucose to 2-deoxy-glucose and 3-*O*-methyl-glucose may be due to compartmental separation of the donor from the acceptors. Furthermore, only a small portion of the isobutyrate fed to the leaves is converted to 1-*O*-isobutyryl-β-glucose. Thus, the concentration of in vivo donor generated by feeding 2 mM isobutyrate (in a total volume of 100 μL/g leaf) is also very low (10–20 μM).

We previously hypothesized a biological role for acyl exchange in regenerating 1-*O*-acyl-β-glucoses during the terminal steps in triacylglucose biosynthesis (Ghangas & Steffens, 1995). The presence of acyl exchange activity in a variety of plants that do not secrete such acylsugars now leads one to believe that the reaction may have other functions as well. The activated 1-*O*-acyl-β-glucoses are perhaps mobilized as diacylglucoses to escape β-glucosidase action. Thus disproportionation and acyl-exchange could provide a mechanism to preserve/regenerate these linkages. Yet another explanation for the entire UDPGlc-dependent pathway is to provide a detoxification mechanism for the plant cell against free fatty acids which are first converted to 1-*O*-acyl-β-glucoses and then to diacylglucoses.

3. Experimental

The reagents for the synthesis of 1-*O*-acyl-β-glucoses have been previously described (Ghangas & Steffens, 1995). 3,4,6-tri-*O*-benzyl-D-glucal was purchased from Aldrich. Dry solvents (methylene chloride, carbon tetrachloride, benzene and toluene) were used as purchased from Aldrich. Ethyl acetate was shaken with anhydrous potassium carbonate (2 g/100 mL), filtered and redistilled from calcium hydride. Preparative silica gel chromatography (Still, Kahn & Mitra, 1978) was performed with silica gel 60 (230–400 mesh or 70–230 mesh, EM Science). Radiochemicals ([U-^{14}C]glucose and [U-^{14}C]2-deoxy-glucose) were purchased from American Radiolabeled Chemicals (St. Louis, MO). Before use [U-^{14}C]-2-deoxy-glucose was purified by TLC over silica gel using $CHCl_3$/MeOH/H_2O (75:22:3). ^{1}H-NMR spectra were recorded with a Varian spectrometer operating at 200 MHz. GC-MS (electron impact) was carried out with a DB-5 column in a Hewlett-Packard 5890 gas chromatograph/5970 mass-selective detector operating at 70 eV (Ghangas & Steffens, 1993). Melting points were determined on an electrothermal melting point apparatus.

1-*O*-lauroyl-β-glucose was prepared from 2,3,4,6-tetra-*O*-benzylglucose and lauroyl chloride in benzene at 62°C as previously described for the synthesis of 1-*O*-isobutyryl-β-glucose (Ghangas & Steffens, 1995; Pfeffer, Rothman & Moore, 1976). 1-*O*-[1-^{14}C-isobutyryl]-β-glucose was converted to 1,*n*-diisobutyryl-β-glucose (1-*O*-[1-^{14}C-isobutyryl],*n*-isobutyryl-β-glucose) using isobutyryl-chloride and pyridine as previously described for the synthesis of donor 1,*n*-dibutyryl-β-glucose (Ghangas & Steffens, 1995). 1-*O*-methyl-α/β-[^{14}C]-glucose was synthesized from [^{14}C]-glucose

according to (Fischer, 1895). 40 μg [^{14}C]-glucose (300 mCi/mmol) was mixed with anhydrous methanol that was made 2% with conc. HCl (1 mL) and kept at 60–64°C for 8 h. The solvent was then removed with a stream of N_2 and the residue purifieded by silica gel chromatography. The synthesis of α- and β- derivatives of 1-*O*-isobutyryl-2-deoxy-glucose is detailed below (Fig. 1). The bold-faced numbers correspond to the structures in Fig. 1. A preliminary report of this work was presented at a recent meeting (Ghangas & Steffens, 1996).

*3.1. 1-O-isobutyryl-2-deoxy-3,4,6-tri-O-benzyl-α-glucopyranose (**2**)*

Isobutyric acid (0.7 mL, 7.5 mmol) was added to a magnetically stirred solution of 3,4,6-tri-*O*-benzyl-D-glucal (**1**) (1.04 g, 2.5 mmol) in anhydrous CH_2Cl_2 followed by triphenylphosphine hydrobromide (Bolitt et al., 1990) ($Ph_3P{\cdot}HBr$)(43 mg, 0.125 mmol). After stirring at ambient temperature for 2 h, the reaction mixture was washed with saturated $NaHCO_3$ and NaCl, dried over anhydrous Na_2SO_4 and concentrated. The residue (1.2 g, 99% yield) was purified by two successive silica gel flash columns using benzene–ethyl acetate (19:1) followed by hexane–EtOAc (8:1). Yield was 0.63 g (52%). R_f 0.18 (hexane–EtOAc: 8:1). ^{1}H-NMR (200 MHz,$CDCl_3$), δ 7.15–7.4 (m, 15H; aromatic ring protons), 6.25 (dd, J = 1.8,1.3 Hz, 1H; α-anomeric), 4.9 (d, J = 10.5 Hz, 1H), 4.45–4.7 (m, 5H), 3.6–4.0 (m, 5H), 2.5 (septet, J = 7 Hz, 1H; $(CH_3)_2CH$), 2.25 (m, 1H), 1.85 (m, 1H), 1.19 (overlapping d, J = 7 Hz, 6H; $(CH_3)_2CH$)

*3.2. 1-O-isobutyryl-2-deoxy-α-glucopyranose (**3**)*

A solution of 1-*O*-isobutyryl-2-deoxy-3,4,6-tri-*O*-benzyl-α-glucopyranose (**2**) (60.6 mg, 0.12 mmol) in anhydrous EtOAc was sparged with dry N_2 and treated with palladium catalyst (Pd on activated carbon, Pd 10%). The suspension was hydrogenated using a Paar apparatus. The hydrogenated product was purified by silica gel flash chromatography using $CHCl_3$–MeOH (7:1). Yield was 23 mg (82%). R_f 0.36 [$CHCl_3$–MeOH (7:1)]. ^{1}H-NMR (200 MHz, acetone-d_6), δ 6.12 (dd, J = 2.2, 1.3 Hz, 1H; α-anomeric), 3.55–3.95 (b, 4H), 3.4 (t, J = 9.1, 1H), 2.55 (sep, J = 7 Hz, 1H; $(CH_3)_2CH$), 2.05 (m, 1H), 1.70 (m, 1H), 1.13 (overlapping d, J = 7 Hz, 6H; $(CH_3)_2CH$).

*3.3. 2-deoxy-2-selenophenyl-3,4,6-tri-O-benzyl-α-glucopyranose (Kaye, Neidle & Reese, 1988; Roush & Lin, 1991) (**4**)*

To a magnetically stirred solution of 3,4,6-tri-*O*-benzyl-D-glucal (**1**) (1.28 g, 3.0 mmol) in dry CCl_4 (10 mL) at −20°C was slowly added dropwise phenylselenenyl chloride (PhSeCl) (1 g, 5.24 mmol dissolved in 4 mL dry CCl_4). The resulting brown–yellow solution was stirred for 60 min at −20°C and then CCl_4 was removed in vacuo. The resulting orange–brown oil was dissolved in THF-H_2O (20 mL, 1:1) and after adding Na_2CO_3 (0.77 g) the mixture was stirred at room temperature for 15 min and then brought to 50°C for 4 h. The mixture was cooled to room temperature, diluted with H_2O and extracted with ether (Et_2O). The ether extract was washed with saturated NaCl and dried over anhydrous $MgSO_4$. The solvent was removed in vacuo and the residue purified by a flash column using two petroleum ether–ethyl acetate solvents (4:1, 1:1). This was followed by a second flash column using hexane–ethyl acetate (7:2). The product was further purified by crystallizing from EtOH. Melting point (uncorrected) 102–103°C. Yield 0.75 g (41%). Rf 0.33 [(petroleum ether–ethylacetate (4:1)]. ^{1}H-NMR (200 MHz,$CDCl_3$), δ 7.56–7.64 (m, 2H; aromatic protons ortho to Se), 7.1–7.4 (m, 18H; aromatic protons), 5.45 (d or dd, J = 2.9–3.17 Hz, 1H; α-anomeric), 5.0 (d, J = 11.4 Hz, 1H), 4.8–4.9 (m, 2H), 4.45–4.65 (m, 3H),4.05–4.2 (m, 2H or 3H), 3.55–3.75 (m, 3H), 3.35 (dd, J = 10.86, 3.21 Hz, 1H)

*3.4. 1-O-isobutyryl-2-deoxy-2-selenophenyl-3,4,6-tri-O-benzyl-β-glucopyranose (**5**)*

A solution of 2-deoxy-2-selenophenyl-3,4,6-tri-*O*-benzyl-α-glucopyranose (**4**) (118 mg, 0.2 mmol), triphenylphosphine (Ph_3P) (78.7 mg, 0.3 mmol), isobutyric acid (25 μL, 0.27 mmol) and 20 mg molecular sieves (4 Å) in dry benzene (1 mL) was prepared. Diethyl azodicarboxylate (Mitsunobu, 1981; Smith, Hale & Rivero, 1986) (DEAD) (52 μL, 0.34 mmol) was slowly added to this solution. The mixture was stirred at 0°C for 30 min and then at room temperature for 3 h. The solvent was removed in vacuo and the crude product was purified by flash chromatography over silica gel using petroleum ether–ethyl acetate (4:1). A second round of flash chromatography using hexanes–ethyl acetate (10:1) was used to further purify product **5**. Yield 61 mg (46%). Rf 0.86 [petroleum ether–EtOAc (4:1)], Rf 0.40 [(hexane–EtOAc(9:1)]. ^{1}H-NMR (200 MHz,$CDCl_3$), δ 7.56–7.64 (m, 2H; aromatic protons ortho to Se), 7.1–7.4 (m, 18H; aromatic protons), 5.72 (d, J = 9.4 Hz, 1H; β-anomer), 4.75–5.0 (m, 3H), 4.45–4.65 (m, 3H), 3.45–3.87 (m, 5H), 3.35 (dd, J = 9.5, 9.4 Hz, 1H), 2.45 (septet, J = 7 Hz, 1H; $(CH_3)_2CH$), 1.20 (overlapping d, J = 7 Hz, 6H; $(CH_3)_2CH$).

3.5. 1-O-isobutyryl-2-deoxy-3,4,6-tri-O-benzyl-β-glucopyranose (6)

A magnetically stirred solution of 1-*O*-isobutyryl-2-deoxy-2-selenophenyl-3,4,6-tri-*O*-benzyl-β-glucopyranose (**5**) (33 mg, 0.05 mmol) in anhydrous toluene (1 mL) and containing catalytic amount of 1,1′-azobis(cyclohexanecarbonitrile (Overberger, Biletch, Finestone, Lilker & Herbert, 1953) (ACHN; Aldrich trade name VAZO®) (1.22 mg, 5 μmol) under dry N_2 gas was prepared. Tributylltin hydride (Bu_3SnH) (67.25 μL, 0.25 mmol) was added to this solution and the reaction stirred at 90°C for 5 h. The solvent was then removed in vacuo and the residue purified by flash chromatography using hexane–ethyl acetate (9:1). Yield was 14 mg (68%). Rf 0.265 [(hexane–petroleum ether (10:1)], Rf 0.187 (hexane–EtOAc (9:1). ^{1}H-NMR (200 MHz, $CDCl_3$), δ 7.15–7.4 (m, 15H; aromatic ring protons), 5.68 (dd, J = 2.2,10 Hz, 1H; β-anomeric), 4.88 (d, J = 10.8 Hz, 1H), 4.48–4.74 (m, 5H), 3.45–3.8 (m, 5H), 2.6 (septet, J = 7 Hz, 1H; $(CH_3)_2CH$), 2.35 (m, 1H), 1.75 (m, 1H), 1.21 (overlapping d, J = 7 Hz, 6H; $(CH_3)_2CH$).

3.6. 1-O-isobutyryl-2-deoxy-β-glucopyranose (7)

A solution of 1-*O*-isobutyryl-2-deoxy-3,4,6-tri-*O*-benzyl-β-glucopyranose (**6**) (20.2 mg, 0.04 mmol) in anhydrous EtOAc (10 mL) was flushed with dry N_2 and treated with palladium catalyst (Pd on activated carbon, Pd 10%). The suspension was hydrogenated using a Paar apparatus (25 psi). The hydrogenated product was purified by silica gel flash chromatography using $CHCl_3$–MeOH (7:1). Rf 0.39 [$CHCl_3$–MeOH (7:1)]; Rf 0.60 [$CHCl_3$–MeOH–H_2O (75:22:3)]. Yield 2.9 mg (31%). ^{1}H-NMR (200 MHz, acetone-d_6), δ 5.7 (dd, J = 10.1, 2.1 Hz, 1H; β-anomeric), 3.45–3.85 (b, 4H), (t, 1H), 2.55 (sep, 1H; $(CH_3)_2CH$), 2.1 (m, 1H), 1.55 (m, 1H), 1.14 (overlapping d, J = 7 Hz, 6H; $(CH_3)_2CH$).

3.7. Enzyme reactions

Foliage samples of greenhouse-grown wheat (*Triticum aestivum*), bean (*Phaseolus vulgaris*) and melon (*Cucumis melo*) were provided by M. E. Sorrells, D. H. Wallace and M. Kyle (Cornell University, Ithaca, NY) and were immediately frozen after harvest. *Lycopersicon pennellii* (LA716), *Lycopersicon esculentum* and *Solanum berthaultii* plants were grown in the greenhouse. *Arabidopsis thaliana* and *Nicotiana tabacum* plants were grown in the laboratory. Leaf extracts were prepared, the proteins partially purified and quantified as described (Ghangas & Steffens, 1995). Anomeric acyl exchange (Ghangas & Steffens, 1993, 1995) was analysed by measuring the transfer of isobutyrate from the synthetic monoisobutyryl donors to [U-^{14}C]glucose, [U-^{14}C]2-deoxy-glucose or 3-*O*-methyl-[U-^{14}C]glucose. Reaction mixes contained 2.5 mM donor, 50 μM acceptor sugar (8×10^4–4.5×10^5 cpm), 50 μg protein in 20 μL (total volume) 50 mM NaPi, pH 6.5. Reaction progress (37°C) was monitored by TLC (Ghangas & Steffens, 1993, 1995). β-glucosidase treatments with Sigma sweet almond enzyme were carried out as previously described (Ghangas & Steffens, 1995).

3.8. Acyl exchange in vivo

Leaves from *L. pennellii* (LA716) plants grown in the greenhouse were washed with EtOH/H_2O system as previously described (Ghangas & Steffens, 1993). After 1 day recovery, the leaves were administered with various combinations of substrates and analysed as previously described (Ghangas & Steffens, 1993).

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