

# GC-MS Characterization of Acetylated $\beta$ -D-glucoopyranosides: Transglucosylation of Volatile Alcohols Using Almond $\beta$ -glucosidase

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$\beta$ -D-Glucoopyranosides of pentan-1-ol, ( $\pm$ )-pentan-2-ol, hexan-1-ol, octan-1-ol, benzyl alcohol, 2-phenylethanol, ( $\pm$ )-2-phenyl-propan-1-ol, 3-phenyl-propan-1-ol, geraniol and nerol were synthesized by transglucosylation of the respective alcohols with cellobiose using almond  $\beta$ -glucosidase. The reaction was carried out in acetonitrile with acetate buffer (vol. ratio 9:1), with the yields 14.4–45.0 %. Transglucosylation was not enantioselective. The products were characterized by GC-MS analysis of prepared tetraacetyl glucosides. Fragment ion characteristics of the aglucone moiety are present in all mass spectra, along with the fragments obtained from acetylated glucose. Acetylated glucosides are separable on HP-101 column (even diastereomeric tetraacetyl  $\beta$ -glucosides of enantiomeric alcohols).

## INTRODUCTION

Alkyl  $\beta$ -glycosides with a short chain length (C4–C9), glycoconjugates of phenylpropane derivatives as well as terpenes belong to naturally occurring glycosides of volatile compounds.<sup>1–3</sup> They are present in plants, mostly in the form of  $\beta$ -O-glycosides. They can be considered as flavour precursors, since their enzymatic/acidic hydrolysis or pyrolysis liberates volatiles.<sup>2,3</sup> Alcohols such as hexan-1-ol, 2-phenyletanol, benzyl alcohol, geraniol and nerol are detected among ubiquitous aglucone moieties.<sup>2</sup> The glycosidically bound volatiles are of interest in the food, cosmetic, tobacco and wine industries.<sup>4–7</sup> They are non-volatile flavouring and fragrance precursors with alternative lasting effects (due to hydrolysis on the skin surface), water soluble transport forms of volatile apolar compounds and some of them posses biological activity.

The use of glycosidases to catalyze the synthesis of alkyl glycosides has attracted considerable interest, since alkyl glycosides are biodegradable non-ionic and non-toxic surfactants.<sup>7–9</sup> Almond  $\beta$ -glucosidase exhibits catalytic activity in the water-organic two-phase system,<sup>10,11</sup> in buffer saturated alcohol-acceptors<sup>12</sup> or in organic solvent with a low water content.<sup>13</sup> Enzymatic glycosylation has the advantage of regio- and often enantioselectivity and may be realized by reverse hydrolysis (condensation) or transfer reaction (transglycosylation).<sup>8</sup> Transglycosylation type reactions often employ glycosyl donors with good leaving groups, such as glycosyl fluorides<sup>14</sup> or *p*-nitrophenyl glycosides.<sup>15</sup> Vulfson *et al.*<sup>16</sup> reported that cellobiose gave alkyl  $\beta$ -D-glycoside through a transfer reaction and that approximately the same yield of glycoside was attained at the same concentrations of glucose when glucose and cellobiose were used as substrates. Vic and

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Thomas<sup>17</sup> found that transglucosylation using cellobiose is optimal in media with 80–90 % (vol. fraction) organic solvent. This enzyme tolerates acetonitrile better than high levels of dimethyl formamide, dimethyl sulfoxide or pyridine.<sup>18</sup> In organic media, enzymatic thermostability may be increased<sup>19</sup> and enzymatic specificity may be changed.<sup>20</sup> Most studies on  $\beta$ -glucosides synthesis have been carried out using almond  $\beta$ -glucosidase in a water-organic two-phase system. The presence of water is necessary to maintain the enzymatic activity but, on the other hand, it causes product hydrolysis, so the water content of the media has to be carefully controlled.<sup>11</sup>

The aim of this study was to investigate transglucosylation of ubiquitous volatile alcohols with cellobiose in acetonitrile with acetate buffer (vol. ratio 9:1) and to characterize the prepared  $\beta$ -D-glucosides after acetylation. Racemic secondary alcohols ( $\pm$ )-pentan-2-ol and ( $\pm$ )-2-phenyl-propan-1-ol were used to investigate the enantioselectivity of almond  $\beta$ -glucosidase transglucosylation. There are no previous reports on the enzymatic enantioselectivity in transglucosylation of racemic alcohols with cellobiose in organic media. Transglucosylation products were, for the first time, characterized by GC-MS analysis of acetylated glucosides. Their detailed gas-chromatographic and mass spectrometric properties were determined in order to investigate the possibility of GC-MS for screening the enzymatic glucoside catalysis.

## MATERIALS AND METHODS

### Materials

Almond  $\beta$ -glucosidase was obtained from Fluka Chemie, Buchs, Switzerland, and was used without further purification. Cellobiose was purchased from Merck, Darmstadt, Germany, and so were silicagel plates (Kieselgel 60, 0.2 mm). Silicagel for flash chromatography (30–60  $\mu$ m) was obtained from Mallinckrodt Baker B.V., Deventer, The Netherlands. Methyl- $\beta$ -glucopyranoside was obtained from Sigma-Aldrich, Taufkirchen, Germany. All other reagents and organic solvents were of the highest grade available commercially from Fluka Chemie, Buchs, Switzerland. Secondary alcohols were used as racemic mixtures.

### Transglucosylation

Cellobiose (0.2 mmol) was dissolved in 0.5 mL acetate buffer (0.2 mol/L; pH = 4.4) and acetonitrile (4.5 mL) was added. One of the glucosyl acceptors (Table I), in four-fold excess (0.8 mmol), was then separately dissolved in the above medium and almond  $\beta$ -glucosidase (10 mg; 5.4 U/mg) was added. The resulting mixture was incubated in sealed vials at 50 °C for 72 hours in a shaking water bath (model SW22, Julabo, Seelbach, Germany). Progress of the reaction was monitored by

TLC. The reaction was stopped by enzyme denaturation (30 min. heating under reflux).

The internal standard methyl  $\beta$ -glucoside was used for quantification. The standard (10 mg) was dissolved in the mixture of dry pyridine (10 mL) and acetic anhydride (15 mL). An aliquot (0.3 mL) was added into 0.1 mL of each reaction mixture for quantification, after acetylation and GC-MS analysis. Acetylation procedure is described in further text. The percentage composition was computed from the GC peak areas. The yield was based on the starting amount of cellobiose.

After enzyme denaturation, the water was removed by azeotropic distillation. Acetonitrile layer was filtered and the precipitate (denaturated enzyme, the remaining cellobiose and glucose) was washed with ethanol and rejected. The combined acetonitrile layer and ethanol extract were evaporated in a rotating evaporator over 0.5 g silicagel for flash chromatography to dryness. Syntheses of all glucosides were carried out in duplicate.

### Flash Chromatography

The glucoside adsorbed on silicagel was then placed on top of a silicagel column (4.5 g). The product was purified by flash chromatography applying the mixture of ethyl acetate and light petroleum (volume ratio,  $\psi = 1 : 1$ ) to remove the unreacted aglycone and the mixture of ethyl acetate and ethanol ( $\psi = 1 : 2$ ) for glucoside elution.

### Thin Layer Chromatography

Prepared glucosides were chromatographed on a thin silicagel layer (TLC). The mixture of ethyl acetate, ethanol and conc. ammonia ( $\psi = 6 : 3 : 1$ ) was used as solvent. After developing the chromatograms, the plates were sprayed with vanillin–sulphuric acid and heated to 120 °C for 10 minutes. Individual glucosides were detected as dark spots.

### Acetylation

Glucosides (10 mg of each) were separately dissolved in dry pyridine (0.4 mL). Acetic anhydride (1 mL) was added and the mixture was stirred at 100 °C. This method is similar to that in an earlier paper.<sup>21</sup> After 1 hour, heating was discontinued, the mixture was cooled at room temperature and 20 mL of cold water with ice was added. Aqueous layer was extracted with 3 x 5 mL ether. Ether extracts were washed with water (3 x 3 mL) and dried over anhydrous MgSO<sub>4</sub>. Combined ether extracts were concentrated to 0.5 mL and 1  $\mu$ L was used for GC-MS analysis.

### Enzymatic Hydrolysis

Purified glucosides (10 mg of each) were separately dissolved in 2 mL of 0.1 mol/L acetate buffer, pH = 5.5. The obtained solution was exhaustively extracted with

pentane (5 x 2 mL). Pentane extract was concentrated to 0.5 mL and no traces of aglucones were detected by GC-MS.  $\beta$ -Glucosidase from almonds (5 mg, 5.4 U/mg) was added to glucosidic solutions along with 3 mL pentane to trap the liberated aglucones. Hydrolysis was carried out for 70 h at 30 °C with the mixture being shaken occasionally, as described in a previous paper.<sup>22</sup> After hydrolysis, the pentane layer was separated, and the remaining aglucone was extracted from the aqueous layer with pentane (3 x 2 mL). The combined pentane extracts were dried over  $\text{Na}_2\text{SO}_4$  and concentrated to the final volume of 0.5 mL, and 1  $\mu\text{L}$  was used for GC-MS analysis.

#### Gas Chromatography–Mass Spectrometry Analysis

Analyses of tetraacetyl glucosides and liberated aglucones were performed on a GC-MS Hewlett Packard (model 5890) with a mass selective detector (model 5971A). GC operating conditions for aglucones: column HP-20M (Carbowax 20M, Hewlett Packard, Vienna, Austria), 50 m x 0.2 mm i.d., film thickness 0.2  $\mu\text{m}$ , column temperature programmed from 70 °C (isothermal for 4 minutes) to 180 °C (4 °C/min) as in our previous paper.<sup>22</sup> GC operating conditions for tetraacetyl glucosides: column HP-101 (Dimethylpolysiloxane, Hewlett Packard, Vienna, Austria), 25 m x 0.2 mm i.d., film thickness 0.2  $\mu\text{m}$ , column temperature programmed from 150 °C (isothermal for 4 minutes) to 220 °C (10 °C/min). Carrier gas: helium, flow rate 1 mL/min; injector temperature: 250 °C; volume injected: 1  $\mu\text{L}$ ; split ratio: 1 : 50. MS conditions: ionization voltage: 70 eV; ion source temperature: 280 °C; mass range: for the aglucones 30–300 mass units and for the tetraacetyl glucosides 30–600 mass units.

## RESULTS AND DISCUSSION

In incubation with cellobiose and glycosyl acceptors, almond  $\beta$ -glucosidase gave glucosides of primary and secondary alcohols with yields of 14.4–45.0 %, Table I. The obtained yields for monoterpene  $\beta$ -glucosides of geraniol and nerol were higher than previously reported.<sup>23,24</sup> The reaction was carried out in acetonitrile with acetate buffer (vol. ratio 9:1), which is reported to be the optimum quantity of water for transglucosylation catalyzed by almond  $\beta$ -glucosidase.<sup>8</sup> The enzymatic activity was not monitored since Vic *et al.*<sup>25</sup> found 75 % residual hydrolytic activity of almond  $\beta$ -glucosidase after 24 h at 45 °C in the same organic media, and even performed 6 days of incubation. The high alcohol concentration (four-fold excess) was used to push the transglucosylation reaction toward glucoside synthesis. The enzymatic process of transglucosylation is controlled by the enzyme ability to transfer the glucosyl moiety from source substrate (cellobiose) to the acceptor,<sup>26</sup> Figure 1.

The liberated molecule of glucose has also the possibility of reacting in a secondary condensation reaction, though transglucosylation is preferred. Unfortunately, a side reaction of liberated glucose and cellobiose is also possible. TLC was used to monitor the increase of product concentration. After the product concentration was almost constant, the reaction was stopped by enzyme denaturation.

The reactivity of various glucosyl acceptors toward cellobiose was observed in the course of this research. Both the transglucosylation rate (monitored by TLC) and yield were higher for primary alcohols than for secondary

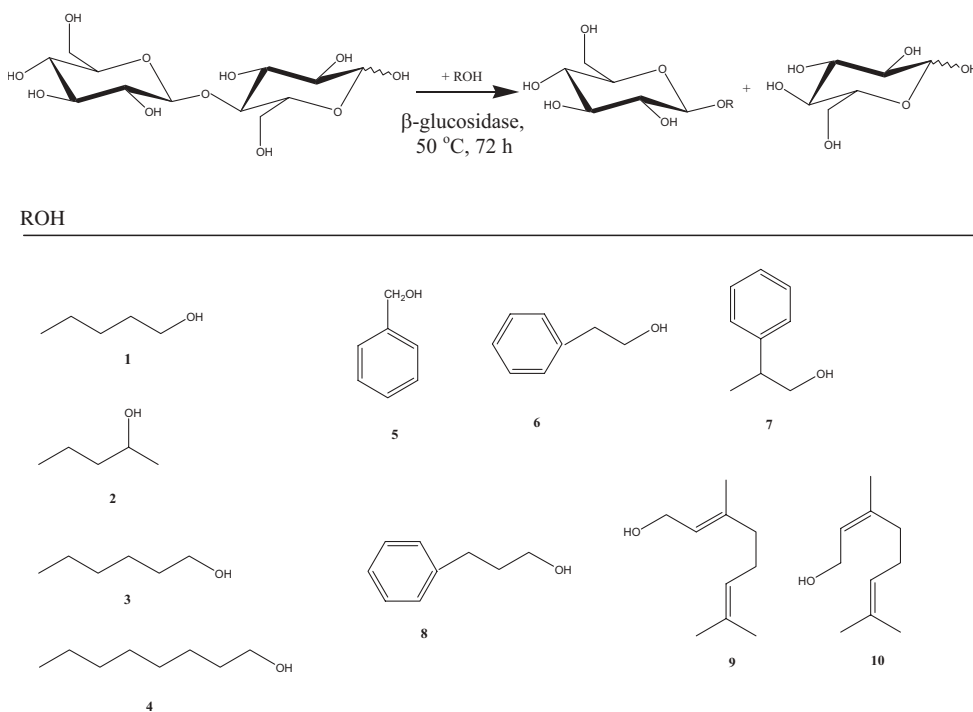


Figure 1. Transglucosylation reaction of cellobiose with aliphatic alcohols using  $\beta$ -glucosidase: **1**, pentan-1-ol; **2**, pentan-2-ol; **3**, hexan-1-ol; **4**, octan-1-ol; **5**, benzyl alcohol; **6**, 2-phenylethanol; **7**, 2-phenylpropan-1-ol; **8**, 3-phenylpropan-1-ol; **9**, geraniol; **10**, nerol.

TABLE I. Yields and  $R_F$  values of  $\beta$ -glucosides obtained by the transglycosylation reaction using  $\beta$ -glucosidase

No.	Glucosyl acceptor	$\beta$ -glucoside	
		Yield / %	$R_F^{(a)}$
1.	pentan-1-ol	45.0	0.34
2.	( $\pm$ )-pentan-2-ol	12.6 <sup>(b)</sup>	0.35 <sup>(c)</sup>
3.	hexan-1-ol	29.4	0.36
4.	octan-1-ol	14.4	0.41
5.	benzyl alcohol	30.6	0.36
6.	2-phenylethanol	30.0	0.35
7.	( $\pm$ )-2-phenyl-propan-1-ol	16.2 <sup>(b)</sup>	0.41 <sup>(c)</sup>
8.	3-phenyl-propan-1-ol	40.2	0.38
9.	geraniol	20.3	0.41
10.	nerol	28.2	0.42

<sup>(a)</sup>  $R_F$  values for solvent system ethyl acetate, ethanol, conc. ammonia, vol. ratio 6 : 3 : 1; solvent front 10 cm on silica gel. Each value is an average from duplicate TLC plates.

<sup>(b)</sup> Yield is expressed as the sum of both diastereomeric glucosides.

<sup>(c)</sup> Two  $\beta$ -diastereomers were not separated by TLC with the applied solvent.

ones as well as for alcohols with a shorter alkyl chain, Table I.  $R_F$  values of prepared glucosides on silicagel plates (TLC) were close (0.34–0.42) with the solvent applied. The enzymatic hydrolysis of purified glucosides with  $\beta$ -glucosidase liberated aglucones, which were analyzed by GC-MS. Since the hydrolysis was performed in acetate buffer with pentane as a trapping agent, the reaction was pushed toward hydrolysis and under these conditions a variety of alcohols could be easily released from the corresponding glucosides.<sup>5,27</sup> The obtained mass spectra and retention times of the liberated aglucones were identical to the spectra and retention times of the starting alcohols.

After the synthesis and chromatographic purification, the obtained glucosides were acetylated and characterized by GC-MS. The prepared tetraacetyl glucosides were adequately separated on a HP-101 column with different retention times (13.88–41.35 min), Table II. GC-analyses indicated that only  $\beta$ -anomers were obtained.

In the discussion about enantioselectivity toward the ( $\pm$ )-alcohols, it should be noted that the carbohydrates are

TABLE II. Retention times and mass spectra of the prepared tetraacetyl- $\beta$ -glucopyranosides

Tetraacetyl- $\beta$ -glucopyranoside of the respective alcohol	Retention time min	Mass spectra $m/z$ (%)
pentan-1-ol	15.30	43 (100), 81 (20), 98 (12), 115 (10), 112 (8), 103 (7), 70 (6), 104 (6), 69 (5), 157 (5), 169 (5), 71 (4), 243 (1)
pentan-2-ol <sup>(a)</sup>	13.88	43 (100), 98 (18), 115 (18), 81 (8), 157 (8), 71 (5), 109 (5), 140 (5), 69 (4), 70 (4), 103 (4), 112 (4), 169 (4), 200 (3), 242 (2), 243 (1), 331 (1)
pentan-2-ol <sup>(a)</sup>	14.35	43 (100), 98 (18), 115 (18), 81 (8), 157 (8), 71 (5), 109 (5), 140 (5), 69 (4), 70 (4), 103 (4), 112 (4), 169 (4), 200 (3), 242 (2), 243 (1), 331 (1)
hexan-1-ol	17.37	43 (100), 112 (46), 98 (32), 157 (32), 185 (30), 115 (20), 146 (24), 141 (22), 81 (20), 169 (18), 200 (18), 287 (14), 242 (8), 210 (4), 331 (4)
octan-1-ol	25.78	43 (100), 81 (22), 98 (14), 112 (9), 69 (8), 115 (8), 70 (7), 103 (6), 71 (5), 109 (5), 157 (5), 140 (4), 145 (4), 169 (2), 243 (1), 242 (1)
benzyl alcohol	27.42	91 (100), 43 (97), 139 (30), 97 (22), 152 (14), 44 (12), 92 (9), 65 (7), 245 (4), 77 (2), 81 (2), 271 (2), 331 (2), 439 ( $M^+$ , 1)
2-phenylethanol	34.68	105 (100), 43 (86), 104 (68), 81 (16), 103 (15), 109 (13), 115 (12), 169 (12), 157 (10), 69 (5), 200 (4), 287 (4), 347 (4) 259 (2), 331 (2), 374 (1)
2-phenyl-propan-1-ol <sup>(a)</sup>	35.28	43 (100), 91 (50), 118 (22), 119 (20), 109 (18), 169 (12), 81 (8), 115 (6), 127 (5), 157 (4), 332 (1), 348 (1)
2-phenyl-propan-1-ol <sup>(a)</sup>	35.64	43 (100), 91 (50), 118 (20), 119 (20), 109 (18), 169 (12), 81 (8), 115 (6), 127 (5), 157 (5), 332 (1), 348 (1)
3-phenyl-propan-1-ol	41.35	43 (100), 91 (46), 118 (24), 81 (17), 44 (14), 169 (14), 98 (10), 109 (10), 116 (10), 331 (4), 200 (2), 373 (1), 286 (1)
geraniol	36.81	43 (100), 69 (75), 68 (32), 81 (23), 109 (23), 41 (20), 93 (18), 169 (12), 136 (8), 139 (7), 211 (1), 245 (1), 331 (1), 427 (1)
nerol	33.13	43 (100), 69 (48), 68 (30), 81 (34), 93 (26), 41 (19), 109 (24), 169 (14), 136 (6), 139 (5), 427 (1), 211 (1)

<sup>(a)</sup> The correct enantiomer of the aglucone moiety was not determined.

chiral and that, consequently, the glycosylation products are diastereomers. The transglucosylation reaction was applied because better enantioselectivity was expected.<sup>8</sup> GC-MS analysis showed that, under the applied reaction conditions,  $\beta$ -glucosidase did not favour exclusively enantioselection, since both alcohols-enantiomers were glucosylated, with different amount ratios. The obtained diastereomeric  $\beta$ -glucosides of racemic alcohols were not separated by TLC with the solvent applied. The racemic mixture of ( $\pm$ )-2-phenyl-propan-1-ol produced through transglucosylation two diastereomeric  $\beta$ -glucosides. After acetylation, these diastereomers were resolved by gas chromatography (amount ratio 1 : 6.6), with different retention time and identical mass spectra. The most reactive enantiomer was not determined. Separation of the diastereomeric tetraacetyl  $\beta$ -glucosides of (+)- and (-)-pentan-2-ol was also obtained (amount ratio 1 : 1.2). Their mass spectra are identical. Both enantiomers of pentan-2-ol showed almost equal reactivity. Glycosylation reactions with no enantioselectivity and acceptable yields are interesting for the resolution of racemic al-

hols in a two-stage process. A racemic alcohol is converted enzymatically to a mixture of diastereomeric glycosides. These diastereomers could be separated by HPLC or converted to acetates and resolved by GC (and further deacetylated). The enzymatic hydrolysis of separated diastereomeric glucosides affords optically pure alcohol.

### Mass Spectra of Acetylated $\beta$ -glucosides

Transglucosylation products were, for the first time, characterized by GC-MS analysis of acetylated glucosides. Acetyl derivatives are suitable for GC separation of diastereomers and could be easily converted into the starting glucosides. In general, GC-MS data of acetylated glycosides are limited compared to the data of glycoside TMS and TFA derivatives.<sup>3</sup>

Mass spectra of all tetraacetyl glucosides contain, among the main signals, acetyl ion ( $m/z = 43$ ) from the acetate group. Further, the spectra contain characteristic aglucone ions and large-fragment ions of pyranose, Figure 2. The ion  $C_5H_5O^+$  ( $m/z = 81$ ) is characteristic of pyrans and is found in all spectra. Fragment ions with large

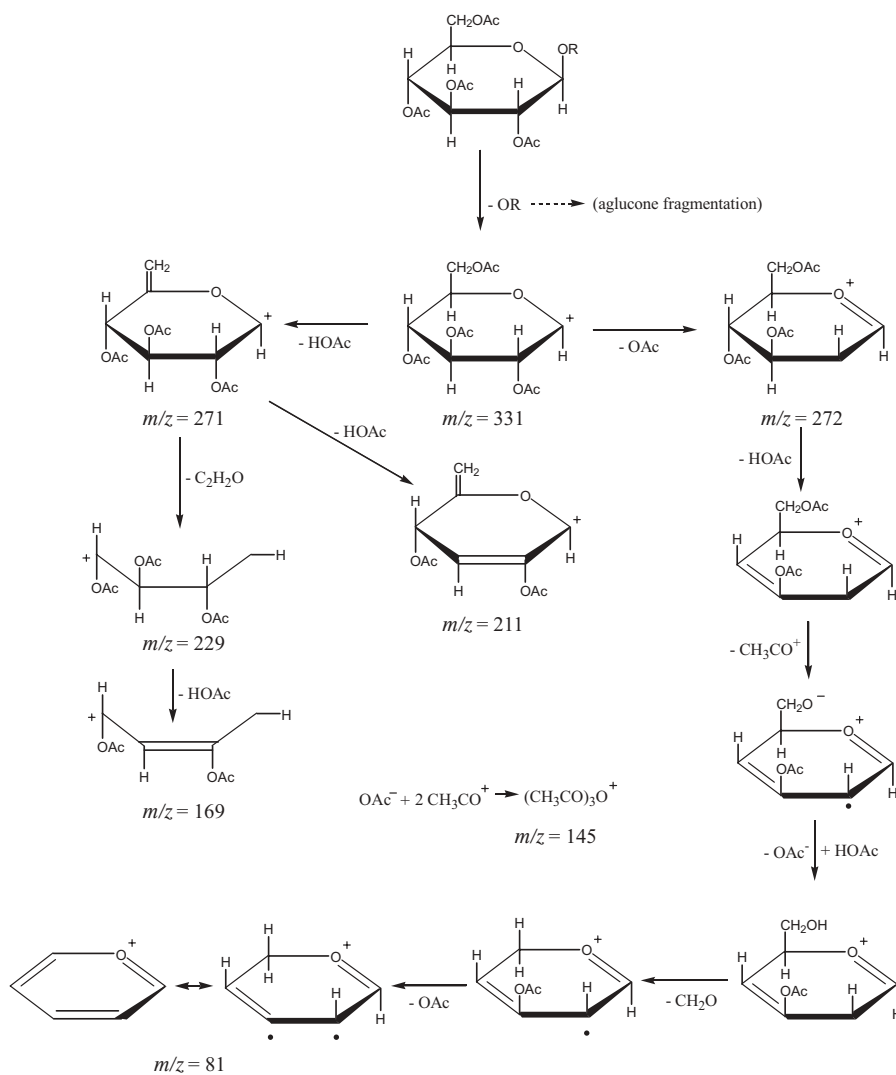


Figure 2. Fragmentation of  $\beta$ -D-glucopyranoside tetraacetates (70 eV), according to the fragmentation of  $\beta$ -D-glucopyranosides.<sup>28</sup>

masses (characteristic of the glucose moiety with acetyl groups) are 331, 271 and 211. The mass 331 is tetraacetyl-glucopyranoside ion, and masses 271 and 211 are generated by elimination of one or two molecules of acetic acid from fragment 331.

The scan of benzyl- $\beta$ -glucopyranoside tetraacetate contains tropylium ion ( $m/z = 91$ ) as the most intensive signal. Tropylium ion originates from the aglucone part. Acetyl ion ( $m/z = 43$ ) is also intensive. Other signals present are 139, 97, 152, 92, 245, 331, 65, 81 and a small signal of the parent ion with 439 mass units. The spectra of 2-phenylethyl- $\beta$ -glucopyranoside tetraacetate contain highly intensive ions  $C_8H_7^+$  ( $m/z = 105$ ) and  $C_8H_8^+$  ( $m/z = 104$ ), characteristic of the aglucone part. The second intensive signal is the acetyl ion. The ion with the largest mass is  $m/z = 347$ , which represents the tetraacetyl-glucopyranoside ion ( $m/z = 331$ ) with one additional oxygen atom, probably from the glucosidic bond. The characteristic of pyran ( $m/z = 81$ ) is also present. The spectra of tetraacetyl- $\beta$ -glucopyranoside of (+)- and (-)-2-phenylpropan-1-ol contain the most intensive ions  $m/z = 43$ , 91, 118 and 169. Acetyl ion is the most intensive signal in the spectra of  $\beta$ -glucopyranoside tetraacetate of 3-phenylpropan-1-ol. Fragments of the aglucone part  $m/z = 91$  and 118 follow in intensity. Ions  $m/z = 104$  and 105 are not as intensive as in the spectrum of 2-phenylethyl- $\beta$ -glucoside tetraacetate. Fragments 331 and 169 are also present.

Mass spectra of tetraacetyl glucosides of pentan-1-ol, (+)- and (-)-pentan-2-ol, hexan-1-ol and octan-1-ol contain the same ions  $m/z = 43$ , 81, 98, 115 and 157 with different intensity. Their retention times are remarkably different, Table II. Characteristic ions originating from the aglucone part are not dominant. Addition of a hydroxyl group to an alkane lowers the ionization energy, but the molecular ion abundance decreases due to decomposition of the ionized hydroxyl group. The abundant  $m/z = 43$  peak, aside from the acetate group, could also be generated in part from  $CHO^+$  and  $C_2H_3O^+$ , which may arise from the  $C_nH_{2n+1}O^+$  ion decomposition.<sup>29</sup> Spectra of the straight chain alcohols-glucosyl acceptors show products that could result from the molecular ion dehydration and are not intensive in the spectra of tetraacetyl glucosides. The  $C_nH_{2n+1}O^+$  ions that could arise from simple  $\alpha$ -cleavage and displacement of the aglucones are of minor importance.

Geraniol and nerol (*cis* and *trans* isomers) have very similar spectra, so the spectra of the corresponding glucoside tetraacetates are also similar. Their retention times were remarkably different, Table II. Besides the most abundant ions from the glucose part, the fragment  $C_5H_9^+$  ( $m/z = 69$ ) is also intensive. This ion originates from the aglucone part. Fragment  $C_9H_{15}O^+$  ( $m/z = 139$ ) is generated by intermolecular cleavage of the methyl group from aglucone. Further elimination of  $C_3H_5$  leads to mass

$m/z = 99$  ( $C_6H_{11}O^+$ ), which by elimination of  $CH_2O$  produces  $C_5H_9^+$ . Ion  $C_3H_5^+$  ( $m/z = 41$ ) could originate from the aglucone by elimination of  $C_2H_4$  from  $C_5H_9^+$ .

## CONCLUSION

$\beta$ -Glucosides were synthesized by transglycosylation in an acetonitrile/acetate buffer system with a low water content (10 %). Further work is needed to increase the yield.  $\beta$ -Glucosidase did not favour exclusively enantioselection, since both alcohols from the racemic mixture were glucosylated, though one enantiomer of 2-phenylpropan-1-ol was considerably more reactive. Product characterization was successfully done by GC-MS analysis of the prepared tetraacetyl glucosides. Fragment ion characteristics of the aglycone moiety were present in all spectra. Acetylated  $\beta$ -glucosides were separable on a HP-101 column, even diastereomeric tetraacetyl  $\beta$ -glucosides of enantiomeric alcohols.

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## SAŽETAK

### GC-MS karakterizacija acetiliranih $\beta$ -D-glukopiranozida: transglukozilacija isparljivih alkohola rabeći $\beta$ -glukozidazu iz badema

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$\beta$ -D-Glukopiranozidi 1-pentanola, ( $\pm$ )-2-pentanola, 1-heksanola, 1-oktanola, benzil-alkohola, 2-feniletanola, ( $\pm$ )-2-fenil-1-propanola, 3-fenil-1-propanola, geraniola i nerola sintetizirani su transglukozilacijom odgovarajućih alkohola rabeći  $\beta$ -glukozidazu iz badema. Reakcija je provedena u acetonitrilu s acetatnim puferom (vol. omjer 9:1), s prinosima 14,4–45,0 %. Transglukozilacija nije bila enantioselektivna. Produkti su karakterizirani GC-MS analizom pripremljenih tetraacetilglukozida. Fragmentni ioni karakteristični za aglukonski dio prisutni su u svim masenim spektrima, pored fragmenata dobivenih iz acetilirane glukoze. Acetilirani glukozidi su odjeljivi na HP-101 koloni (čak i dijastereomerni tetraacetil- $\beta$ -glukozidi enantiomernih alkohola).