Synthesis of the Inhibitors of Glycanases

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2,3-Epoxypropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside was obtained by oxidation of allyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside; deacetylation of the former compound afforded 2,3-epoxypropyl β-D-glucopyranoside. Analogous oxidation and deacetylation of allyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside produced 2,3-epoxypropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside and 2,3-epoxypropyl β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside, respectively. Structure, anomeric and absolute configuration of these substances were determined by ^{1}H and ^{13}C NMR spectroscopic methods, and mass spectrometric methods. The deacetylated epoxyalkyl β-D-glycosides were effective as irreversible active-site directed inhibitors of β-glycosidase from sweet almonds.

Epoxyalkyl β-D-glycosides of mono- and oligosaccharides have been reported [1—7] to be irreversible inhibitors attacking the active centre of glycosidases. 2,3-Epoxypropyl β-D-glucopyranoside (*III*) was first prepared by *Barnett* and *Ralph* [4], who have characterized this sirup by specific rotation only. *Rodriguez* and *Stick* [5] have reported only partial ^{1}H and ^{13}C NMR data concerning the structure of glycoside *III* and 2,3-epoxypropyl β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (*VI*).

Compounds *III* and *VI* can be obtained from allyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (*I*) or alternatively, from allyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (*IV*) by oxidation with 4-nitroperoxybenzoic [2] or 3-chloroperbenzoic acids [4, 5, 7] to form 2,3-epoxypropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (*II*) and 2,3-epoxypropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (*V*), respectively, and by their subsequent deacetylation.

Oxidation of compounds *I* and *IV* gave rise to a new centre of chirality thus producing the corresponding diastereoisomers which have not been separated as yet [5]. *Rodriguez et al.* [6] have obtained optically pure epoxyalkyl-β-D-glucosides by asymmetric synthesis and determined their structures by ^{13}C NMR spectroscopy.

This paper presents unanimously the structure, anomeric configuration, and conformation of compounds *I*—*VI* and absolute configuration of (*R,S*)-diastereomers *II*, *III*, *V*, and *VI*.

Synthesis of the glycoside *I* has been described in several papers [2, 4, 5, 7, 8], but the compound has been characterized only partially by ^{13}C NMR method. The glycoside *I* was in the present work unambiguously characterized by ^{1}H and ^{13}C NMR methods (Table 1).

Values $J_{1,2}$ and $J_{2,3}$ of compound *I* (7.8 and 9.5 Hz, respectively) indicated a trans-diaxial arrangement of the corresponding protons, evidencing therefore the β-anomeric configuration. The high values $J_{1,2}$ to $J_{4,5}$ are indicative of $^4C_1$ conformation of this compound.

The epoxide *II* was obtained by oxidation with peroxyphthalic acid. The coupling constant values (Table 1) evidenced that this compound possesses a β-anomeric configuration and a $^4C_1$ conformation. The absolute configuration of both isomers could be deduced from ^{13}C NMR spectral data by analogy with those published [6] for either (*R*) isomer (δ = 100.8 (C-1), 69.0 (C-7), 50.0 (C-8), and 43.9 (C-9)) or (*S*) isomer (δ = 100.4 (C-1), 70.6 (C-7), 50.4 (C-8), and 43.9 (C-9)). As seen, these values harmonize with those presented in Table 1. The ^{13}C NMR spectrum of epoxide *II* disclosed the crystalline mixture of both diastereoisomers to contain approximately equal amounts of (*R*) and (*S*) components.

Deacetylation of the acetylated epoxide *II* with sodium methoxide afforded epoxide *III* having the same anomeric configuration and conformation as compounds *I* and *II*. It is worth noting that only a very small amount of sodium methoxide had to be used for deacetylation of the compound *II* to avoid its partial decomposition. The ^{13}C NMR data provided an evidence for the assignment of the absolute configuration of both isomers of the epoxide *III* analogously

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Table 1. NMR Data of Glucosides *I*—*III*

Compound			Chemical shift, δ										
			1	2	3	4	5	6	6′	7	8	9	OAc
I		H	4.54 (7.8)	5.00 (9.5)	5.20 (9.8)	5.07 (9.5)	3.67 (4.6, 2.6	4.25 12.3)	4.12	4.32, 4.08	5.83 (m)	5.22	2.12, 2.08 2.05, 2.04
		C	99.56	71.33	72.90	68.49	71.82	61.98		70.04	133.34	117.65	20.63, *
II	(*R*)	H	4.57 (7.9)	5.02 (9.5)	5.21 (9.5)	5.08 (9.5)	3.72 (5.0, 2.4	4.25 12.3)	4.15	3.86	3.15 (m)	2.70, 2.65 (2.6, 5.2)	2.09, 2.07 2.03, 2.01
		C	100.94	71.14	72.65	68.31	71.84	61.80		69.08	50.10	44.03	20.51—20.64
	(*S*)	H	4.66 (7.9)	5.00 (9.5)	5.22 (9.5)	5.10 (9.5)	3.72 (5.0, 2.4	4.25 12.3)	4.15	4.06; 3.49 (2.9, 6.7, 11.9)	3.15 (m)	2.70, 2.56 (2.6, 4.7)	
		C	100.40	71.14	72.70	68.31	71.84	61.8		70.57	50.40	43.96	
III	(*R*)	H	4.48 (6.6)	3.28 (8.4)	3.48 (8.9)	3.37 (x)	3.45 (1.7, 5.6	3.90 12.2)	3.70	4.08, 3.58 (2.4, x, 12.2)	3.38 (m)	2.80, 2.95 (x)	
		C	103.28	74.01	76.58	70.51	76.87	61.62		71.27	52.37	46.01	
	(*S*)	H	4.51 (6.5)	3.28 (8.4)	3.48 (8.9)	3.37 (x)	3.45 (1.7, 5.6	3.90 12.2)	3.70	4.23, 3.72 (2.4, 6.9, 12.2)	3.38 (m)	2.80, 2.95 (x)	
		C	103.26	74.01	76.58	70.51	76.87	61.62		71.40	52.60	45.95	

(*R*) and (*S*) – isomers formed at epoxy side chain [1]; H, C – nucleus observed; coupling constants are given in parentheses; x – not assigned or not resolved, m – multiplet, * – chemical shifts of the carbonyl group: *I* – 170.67, 170.29, 169.32, *II* – 170.32, 170.13, 169.33.

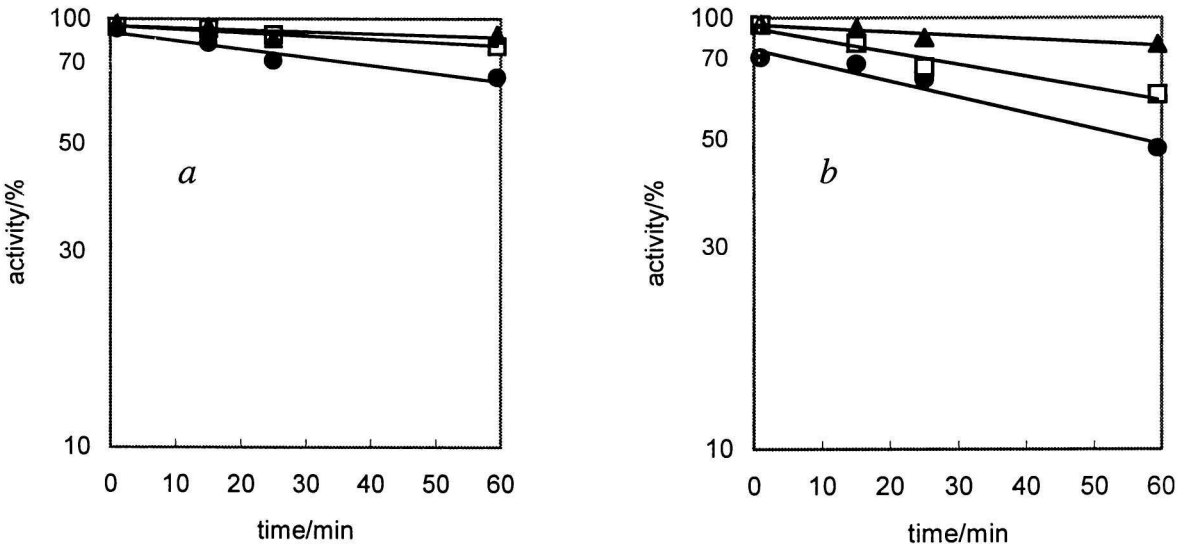


Fig. 1. Deactivation of sweet almond β-glucosidase by epoxypropyl β-D-glucopyranoside (*a*) and epoxypropyl β-cellobioside (*b*). The concentrations of the respective inhibitors were: 10^{-4} mol dm^{-3} (▲), 10^{-3} mol dm^{-3} (□), 10^{-2} mol dm^{-3} (●).

as in the case of the epoxide *II* (Table 1).

Although preparation of the glycoside *IV* has already been reported [2, 5, 8—10], still its characterization by ^{1}H and ^{13}C NMR spectra was equivocal. Data presented in Table 2 showed that the glycoside *IV* has a β-anomeric configuration and $^4C_1$ conformation and that the same anomeric configuration and conformation belong to compounds *V* and *VI*.

Analogous oxidation with monoperphthalic acid employed with glycoside *II* was applied for oxidizing compound *IV* to glycoside *V*. A new centre of chirality created by this reaction gave rise to (*R*) and (*S*) diastereoisomers, the absolute configurations of which were determined by means of ^{13}C NMR analysis as already mentioned with the glycoside *II* (Table 2).

The epoxide *VI* was produced by deacetylation of glycoside *V*. Anomeric configuration, conformation, and absolute configuration of both isomers were evident from ^{1}H and ^{13}C NMR data (Table 2).

Both 2,3-epoxypropyl β-D-glucopyranoside and

Table 2. NMR Data of Cellobiosides

Compound			Chemical shift, δ										
			1	2	3	4	5	6	6′	7	8	9	OAc
IV	A	H	4.51 (7.9)	4.93 (9.7)	5.14 (9.7)	5.06 (9.7)	3.66 (4.6, 2.6	4.37 12.3)	4.04				
	B		4.51 (7.9)	4.92 (9.7)	5.18 (9.7)	3.78 (9.7)	3.58 (4.6, 2.6	4.52 12.3)	4.09	4.30, 4.08 (4.9, x, 13.2)	5.84 (m)	5.25	1.98—2.13
	A	C	100.76	71.62	72.66	67.81	71.96	61.56					
	B		99.38	71.55	72.93	76.58	72.53	61.86		70.02	133.32	117.64	20.63, *
V	A	H	4.52 (7.9)	4.92 (9.2)	5.15 (9.2)	5.06 (9.2)	3.67 (4.4, 2.6	4.37 12.4)	4.05				
	B (*R*)		4.52 (7.9)	4.90 (9.2)	5.17 (9.2)	3.79 (9.2)	3.62 (x, 5.4	4.54 11.3)	4.10	3.80	3.12 (m)	2.78, 2.62 (2.6, 5.1)	2.01—2.09
	(*S*)		4.52 (7.9)	4.90 (9.2)	5.17 (9.2)	3.79 (9.2)	3.62 (x, 5.4	4.54 11.3)	4.10	4.00; 3.47 (x)	3.12 (m)	2.78, 2.54 (2.6, 4.9)	
	A	C	100.77	71.65	72.83	67.84	72.01	61.78					
	B (*R*)		100.92	71.53	72.96	76.42	72.44	61.60		69.50	50.50	44.10	20.56—20.90, *
	(*S*)		100.36	71.53	72.96	76.46	72.44	61.60		70.58	50.24	44.18	
VI	A	H	4.49 (7.8)	3.30 (9.2)	3.48 (x)	3.39 (x)	3.47 (2.0, 6.0	3.90 12.2)	3.71				
	B (*R*)		4.50 (7.8)	3.33 (9.2)	3.62 (x)	3.64 (x)	3.57 (1.0, 4.6	3.96 12.4)	3.79	4.09, 3.71 (2.5, 6.0, 12.2)	3.38 (m)	2.95, 2.80 (3.0, 4.4)	
	(*S*)		4.53 (7.8)	3.33 (9.2)	3.62 (x)	3.64 (x)	3.57 (1.0, 4.6	3.96 12.4)	3.79	4.23, 3.58 (x)	3.38 (m)	2.95 2.80 (3.0, 4.4)	
	A	C	103.47	74.07	76.40	70.37	76.89	61.48					
	B (*R*)		103.11	73.79	75.14	79.49	75.70	60.90		71.42	52.59	46.01	
	(*S*)		103.11	73.79	75.14	79.49	75.70	60.90		71.36	52.37	46.01	

A – Glc^{II} unit, B – Glc^{I} unit; (*R*) and (*S*) – isomers formed at epoxy side chain [1]; H, C – nucleus observed; coupling constants are given in parentheses; x – not assigned or not resolved, m – multiplet, * – chemical shifts of the carbonyl group: *IV* – 169.03—170.48, *V* – 169.05—170.52.

Table 3. First-Order Rate Constants for the Deactivation of Sweet Almond β-Glucosidase by Epoxyalkyl Glucoside and Cellobioside at pH 5.5 and 37 °C

Inhibitor concentration / mol dm^{-3}	$k \cdot 10^{-3}$/min 10^{-2}	10^{-3}	10^{-4}
Epoxyalkyl-β-D-glucoside	359	203.8	97.4
+ cellobiose [0.01 mol dm^{-3}]		44.3	
Epoxyalkyl-β-D-cellobioside	551	493	202
+ cellobiose [0.01 mol dm^{-3}]		61.3	

Table 4. CI (Pyridine) Mass Spectral Data of Compounds *IV* and *V*

IV		*V*		Type of ions
m/z	I_r/%	*m/z*	I_r/%	
756	100	772	100	$[M + Pyridine\ H]^+$
331	24	331	27	$[B_1]^+$
410	0.2	410	3	$[B_1 + Pyridine]^+$
329	5	345	45	$[Z_1]^+$
408	0.1	424	3	$[Z_1 + Pyridine]^+$

2,3-epoxypropyl β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside irreversibly inactivated β-glucosidase from sweet almonds (Fig. 1). First-order rate constants for the deactivation were higher for the cellobiosyl than for the β-D-glucopyranosyl derivative (Table 3). The loss of β-glucosidase activity was prevented in the presence of an excess of cellobiose (0.01 mol dm^{-3}) indicating that the inactivation was directed against the active centre of the enzyme.

Electron ionization (EI) and chemical ionization with pyridine as reagent gas (CI (pyridine)) mass spectra of compound *V* are illustrated in Fig. 2. Assignment of the CI spectra above $m/z = 329$ is shown in Table 4. The molecular ions absented in the EI mass

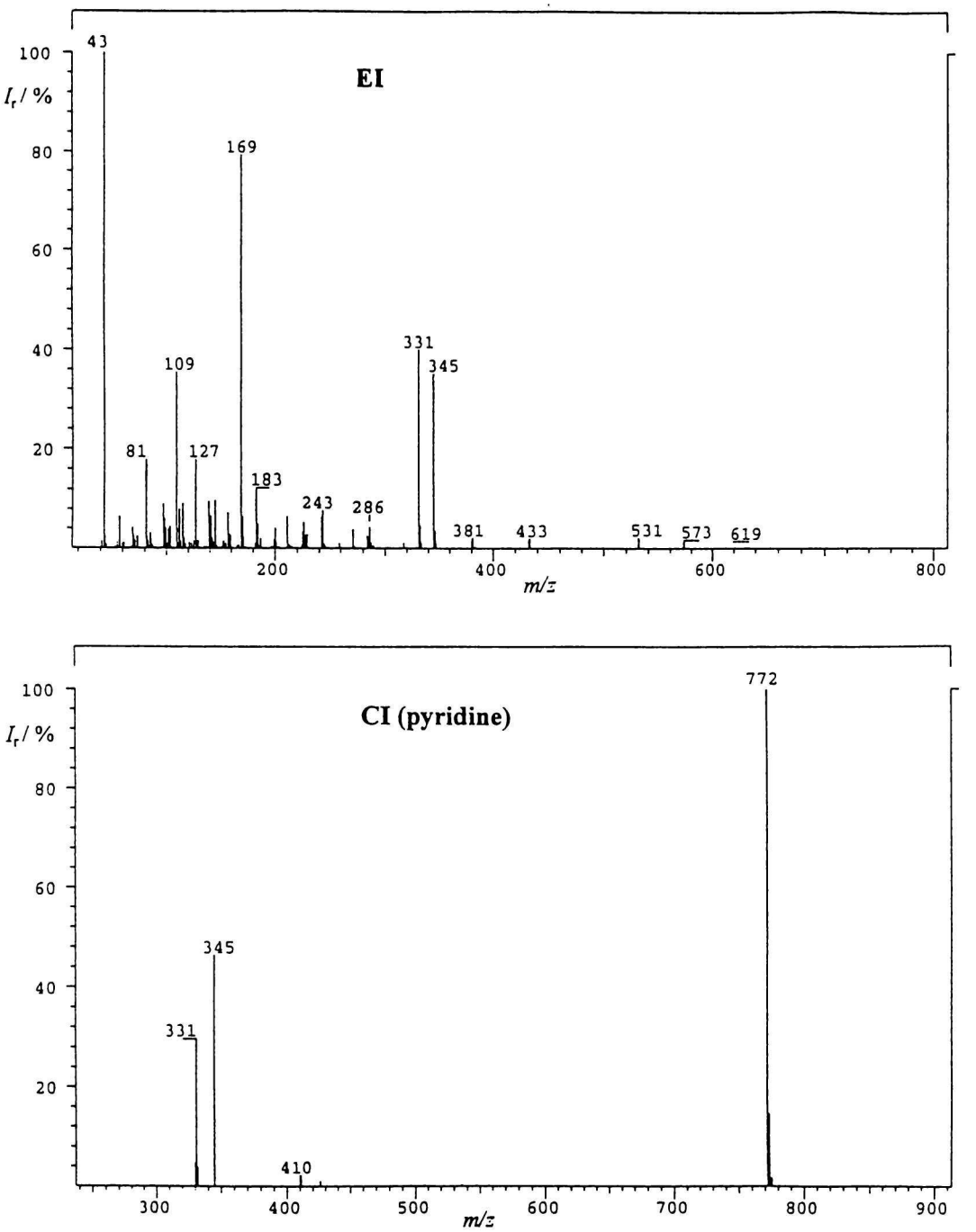


Fig. 2. Electron impact (EI) and chemical ionization (CI) mass spectra of 2,3-epoxypropyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (*V*).

spectra and the most prominent recognizable ions were formed by the loss of 119 mass units $[M + H - 2AcOH]^+$ The molecular mass was determined under CI conditions with protonated pyridine as a reagent gas [11]; observed were also ions $[B_1 + Pyridine]^+$ and $[Z_1 + Pyridine]^+$ originating from ion-molecule reactions between B_1 or Z_1 type of the sample ions and reagent gas molecules as well [12, 13].

EXPERIMENTAL

Melting points were determined on a Kofler micro hot-stage. Solutions were evaporated under diminished pressure at 30—40 °C. Compounds *I* and *IV* were prepared according to [2]. Thin-layer chromatography was conducted on silica gel sheets (Kieselgel 60, Merck) in $\varphi_r = 7\ \ 3$ (S_1) and $\varphi_r = 1\ \ 1$ (S_2) benzene—ethyl acetate, respectively. Preparative

chromatography was performed in columns (120 × 1.5 cm) of silica gel (0.04—0.1 mm) eluted with solvent S_2. The NMR spectra were recorded at 25 °C on an FT NMR AVANCE DPX 300 spectrometer (^{1}H at 300.13 MHz and ^{13}C at 75.46 MHz) equipped with selective excitation unit and gradient enhanced spectroscopy kit (GRASP) for generation of z-gradients up to 50 Gauss/cm^{-1} in a 5 mm inverse probe. Acetylated samples were dissolved in $CDCl_3$ and deacetylated ones in deuterium oxide containing TMS ($\delta = 0.0$) or acetone ($\delta = 2.22$ for ^{1}H and $\delta = 31.07$ for ^{13}C) as internal reference substances. Following techniques were used for assignment of the respective signals: DEPT [10], 2D DQF COSY with pulsed field gradients [14], and ^{1}H detected heterocorrelated HSQC experiments [15] with composite GARP sequence decoupling with 3-9-19 pulse sequence with pulsed field gradients for water suppression [16].

EI and CI (pyridine) mass spectra were measured with a Finnigan MAT SSQ 710 instrument (direct inlet probe, 70 eV, 200 μA, source temp. 150 °C, reagent gas pressure 190 Pa).

2,3-Epoxypropyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranoside (*II*)

The compound *I* (2 g; 5.1 mmol) dissolved in dichloromethane (60 cm^3) containing monoperphthalic acid [17] (2.81 g; 15.4 mmol) was refluxed for 6 h and cooled to 0 °C. Phthalic acid was filtered off and the filtrate was washed stepwise with 0.5 M-$KHCO_3$ (2 × 100 cm^3) and water (2 × 100 cm^3). The organic layer was dried ($MgSO_4$), the dichloromethane solution was concentrated and the residue was analyzed by TLC (S_1). The crude material contained, in addition to epoxide *II* also the unreacted glycoside *I*. Pure compound *II* (1.2 g, 57.6 %), m.p. = 106 °C, [α](D, 20 °C, ρ = 10 g dm^{-3}, chloroform) = −20°, was obtained by separation on silica gel (S_2). Ref. [5] reports m.p. = 115—117 °C, [α](D, 20 °C, ρ = 10 g dm^{-3}, chloroform) = −18°

2,3-Epoxypropyl β-D-Glucopyranoside (*III*)

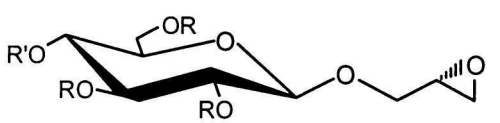
A 1 M-$NaOCH_3$ solution (0.05 cm^3) was added to a stirred solution of the acetylated epoxide *II* (50 mg; 0.12 mmol) in anhydrous MeOH (1 cm^3) at room temperature. After 25 min the solution was neutralized by Amberlite (1RC-50, H^+), filtered and the filtrate was concentrated to give the sirupy epoxide *III* (25 mg, 85 %), [α](D, 20 °C, ρ = 10 g dm^{-3}, MeOH) = −32°; Ref. [4] reports [α](D, 20 °C, ρ = 1 g dm^{-3}) = −35.8°

2,3-Epoxypropyl 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (*V*)

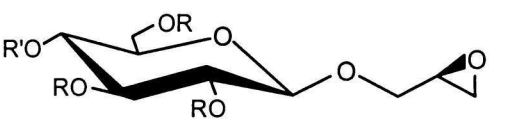
A solution of glycoside *IV* (0.48 g; 0.7 mmol) in dichloromethane (7.5 cm^3) containing monoperphthalic acid (300 mg; 1.65 mmol) was refluxed for 4 h, cooled to 0 °C, filtered and the filtrate was washed successively with 0.5 M-$KHCO_3$ (2 × 20 cm^3) and water (2 × 20 cm^3). The organic layer was dried, concentrated and the residue was analyzed by TLC (S_2). Compound *V* was obtained by separation over a silica gel-packed column (S_2). Yield 330 mg (67 %), m.p. = 190 °C, [α](D, 20 °C, ρ = 10 g dm^{-3}, chloroform) = −23° Ref. [5] reports m.p. = 190—192 °C, [α](D, 20 °C, ρ = 10 g dm^{-3}, chloroform) = 19.2°

2,3-Epoxypropyl β-D-Glucopyranosyl-(1→4)-β-D-glucopyranoside (*VI*)

A solution of epoxide *V* (60 mg; 0.15 mmol) in an-

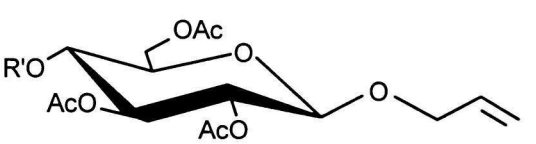


(*R*) isomer



(*S*) isomer

II R = R′ = Ac
III R = R′ = H
V R = Ac, R′ = β-D-Glc*p*(OAc)$_4$
VI R = H, R′ = β-D-Glc*p*



I R′ = Ac
IV R′ = β-D-Glc*p*

hydrous MeOH (1 cm^3) treated with 1 M-CH_3ONa (0.07 cm^3) was stirred at ambient temperature for 30 min. The title product was obtained as a sirup by the same procedure as described with the epoxide *III*. Yield 30 mg (86 %), $[\alpha](D, 20°C, \rho = 1\ g\ dm^{-3}$, MeOH) = −17.0°

Deactivation of β-Glucosidase

Sweet almond β-glucosidase (Serva, 10 μg) was incubated in the presence of various concentrations of the corresponding epoxypropyl glycoside in a total volume 0.02 cm^3 of 0.05 mmol dm^{-3} citrate—phosphate buffer at 37°C for various time intervals. At the end of the preincubation, 0.43 cm^3 of the buffer containing 25 μg of 4-nitrophenyl β-D-glucopyranoside were added to each mixture and the incubation was continued for another 1 h at 37°C. The reaction was terminated by addition of 2.5 cm^3 of 4 % Na_2CO_3 and the absorbance was measured at $\lambda = 410$ nm. Logarithms of the relative activities were plotted against time of inactivation and the first-order rate constants for the deactivation were calculated using a Sigmaplot computer program (Jandel Co.).

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REFERENCES

1. Thomas, E. W., *Carbohydr. Res. 13*, 225 (1970).
2. Legler, G. and Bause, E., *Carbohydr. Res. 28*, 45 (1973).
3. Legler, G., *Adv. Carbohydr. Chem. Biochem. 48*, 319 (1990).
4. Barnett, J. E. G. and Ralph, A., *Carbohydr. Res. 17*, 231 (1971).
5. Rodriguez, E. B. and Stick, R. V., *Aust. J. Chem. 43*, 665 (1990).
6. Rodriguez, E. B., Scally, G. D., and Stick, R. V., *Aust. J. Chem. 43*, 1391 (1990).
7. Bessell, E. M. and Westwood, J. H., *Carbohydr. Res. 25*, 11 (1972).
8. Chernyak, A. Ya., Antonov, K. V., and Kochetkov, N. K., *Carbohydr. Res. 141*, 199 (1985).
9. Lee, R. T. and Lee, Y. C., *Carbohydr. Res. 37*, 193 (1974).
10. Bendall, M. R., Dobbrell, D. M., and Pegg, D. T. *J. Am. Chem. Soc. 103*, 4603 (1981).
11. Pätoprstý, V., Kováčik, V., and Karácsony, Š., *Rapid Commun. Mass Spectrom. 9*, 840 (1995).
12. Kováčik, V. and Grützmacher, H. Fr., *Org. Mass Spectrom. 25*, 687 (1990).
13. Kováčik, V., Hirsch, J. Thölman, D., and Grützmacher, H. Fr., *Org. Mass Spectrom. 26*, 1085 (1991).
14. Davies, L. A., Laue, E. D., Keeler, J., Moskau, D., and Lohman, J., *J. Magn. Reson. 94*, 637 (1991).
15. Schleucher, J., Schwendinger, M., Sattler, M., Schedletzky, O., Glaser, S. J., Sorensen, O. W., and Griesinger, C., *J. Biomol. NMR 4*, 301 (1994).
16. Sklenar, V., Piotto, M., Leppik, R., and Saudek, V., *J. Magn. Reson., Ser. A 102*, 241 (1993).
17. Böhme, H., *Org. Synth. Coll. Vol. III*, p. 619.