J. Appl. Glycosci., **51**, 197−202 (2004)

Enzymatic Syntheses of Cycloalkyl β-D-Glucopyranosides and Their Inhibition Activity for Plant β-Glucosidase

(Received December 8, 2003 ; Accepted February 22, 2004)

Masayasu Takada* and Koichi Ogawa*,**

Nihon Shokuhin Kako Co., Ltd. (30, Tajima, Fuji 417−8530, Japan)

Abstract: Cycloalkyl β-D-glucopyranosides were synthesized by transglucosylation of a β-glucosidase from fungi. When cyclopropanemethanol (CPAM), cyclopentanol (CPE) and cyclopentanemethanol (CPEM) were used as the acceptors, the enzyme stereoselectively synthesized cyclopropylmethyl, cyclopentyl and cyclopentylmethyl β-D-glucopyranosides (abbreviated to CPAM-β-G, CPE-β-G and CPEM-β-G, respectively) from cellobiose as a glucosyl donor. Among the above three β-glucopyranosides, only CPEM-β-G was found to inhibit sweet almond enzyme activity (K_i = 0.15±0.02 mM). Other cycloalkyl β-D-glucopyranosides had little/or no inhibitory activity toward the β-glucosidases examined. CPEM itself had a weak inhibitory activity for sweet almond enzyme, with an uncompetitive type. However, the introduction of a glucose molecule to CPEM as a glycon converted its inhibition type into a competitive one. The K_i value of CPEM-β-G for the enzyme was reduced to about 1/7 compared with the corresponding cyclic alcohol. As it is expected that CPEM-β-G is much more likely to inhibit the activity of an enzyme from plant origin compared with those from fungi, we studied the possibility of CPEM-β-G inhibiting the activities of β-glucosidases extracted from the cut flowers on the basis of their kinetic data.

Key words: cycloalkyl β-D-glucopyranosides, β-glucosidase inhibitor, transglucosylation, cut flowers, cyclopentylmethyl β-D-glucopyranoside

Various models have been proposed for the reaction mechanism of glycosylase in the transition state,[1−4] but a reasonable model remains to be established. The oxocarbonium ion model has been applied to interpret the reaction mechanism of many glycosylases such as lysozyme,[5,6] glucoamylase[7] and glucosidase.[8] In this mechanism, when the glycosidic bond is cleaved, a glycosyl oxocarbonium ion intermediate is formed for catalysis. Evidence of the role of the transition state intermediates came from inhibition studies involving validamine,[9] N-substituted valiolamine derivatives,[10] acarbose,[11] nojirimycin,[12] 1-deoxynojirimycin[13] and N-substituted derivatives of 1-deoxynojirimycin.[14] These inhibitors have been designed, synthesized and purified from plant extracts or the culture broth of microorganisms as the analogues of the charge and/or the shape of the oxocarbonium ion-like transition state intermediates.[15] They have an easily pro-tonated basic nitrogen atom in the position of the anomeric oxygen or the ring oxygen atom. However, the incorporation of a nitrogen positive center into a pyranose ring requires a relatively long reaction sequence.[16]

As its structural similarity with the oxocarbonium ion-like transition state intermediates, many researchers have performed their inhibition studies on glycosylases using glucono-1,5-lactone.[17−19] This compound is a relatively strong inhibitor toward β-glucosidases and has no nitrogen atom in a pyranose ring. However, it has been pointed out that their tendency to undergo hydrolytic ring opening under mildly basic conditions and the conversion into glucono-1,4-lactone (inactive) are the great disadvantage of the measurement of inhibition constants.[18,19] We therefore attempted to prepare more convenient β-glucosidase inhibitors by enzymatic method, which have rather simple chemical structures without a nitrogen atom in the position of the anomeric oxygen or the ring oxygen atom, and are quite stable in an aqueous solution.

MATERIALS AND METHODS

Materials. Cyclopropanemethanol (CPAM) was purchased from Kanto Kagaku Co., Ltd. (Japan). Cyclopentanol (CPE) and cyclopentanemethanol (CPEM) were obtained from Lancaster Co., Ltd. (England). *p*-Nitorophenyl β-glucopyranoside (pNP-β-G) was a product of Nihon Shokuhin Kako Co., Ltd. (Tokyo). The highly purified β-glucosidases from *Aspergillus niger* and *Trichoderma viride* were generous gifts from Dr. T. Unno, Nihon Shokuhin Kako Co., Ltd. (Japan) and Dr. G. Okada, Shizuoka University (Japan), respectively. The purified sweet almond β-glucosidase was purchased from Sigma Aldrich (USA). These purified β-glucosidases were used for the inhibitory studies. On the other hand, β-glucosidases from *Trichoderma reesei* (Cellcrust) and *A. niger* (Novozyme 188) were obtained from Novo Nordisk A/S, and the enzymes from *T. viride* (Meicelase) and sweet almond (BGH-101) were purchased from Meiji Seika Kaisha, Ltd. (Japan) and Toyobo Co., Ltd. (Japan), respectively. These enzymes were used for the preparation of cycloalkyl β-D-

* Contributed equally to this work.
** Corresponding author (Tel. +81−54−553−5995, Fax. +81−54−553−2986, E-mail: koichi.ogawa@nisshoku.co.jp).
Abbreviations: CPAM, cyclopropanemethanol; CPE, cyclopentanol; CPEM, cyclopentanemethanol; CPAM-β-G, cyclopropylmethyl β-D-glucopyranoside; CPE-β-G, cyclopentyl β-D-gluco-pyranoside; CPEM-β-G, cyclopentylmethyl β-D-gluco-pyranoside; pNP-β-G, *p*-nitrophenyl β-D-glucopyranoside; IC_{50}, concentration of the inhibitor required to cause 50% inhibition.

glucopyranosides without further purification. Other chemicals were of the highest quality available commercially.

Analytical methods. HPLC analyses for cycloalkyl β-D-glucopyranosides were performed on a YMC column AQ-312 ODS (6×150 mm) or Shodex Asahipak column GS-220 HQ (7.5×300 mm) in a Hitachi 6000-series liquid chromatograph equipped with a Hitachi L3350 RI monitor. Both columns were eluted with distilled water. The operating temperature of the former column was at 40°C and that of the latter was at 65°C, and the flow rate was 1.0 and 0.6 mL/min, respectively.

^{13}C- and ^{1}H-NMR spectra were obtained with a JEOL JNM-EX 270 spectrometer operating at 68 MHz in the pulsed fourier-transform mode with complete proton decoupling, and 270 MHz, respectively. Chemical shifts are expressed in ppm relative to sodium 3-(trimethylsilyl) propanesulfonate sodium salt (TPS) as an internal standard.

Enzyme assays. β-glucosidase activity was determined by the following method: the reaction mixture (1.0 mL) comprising 0.05–6.0 mM of pNP-β-G as a substrate in 50 mM sodium acetate buffer (pH 5.0) was preincubated at 30°C for 10 min. The reaction was performed with appropriately diluted β-glucosidase in the presence of 0–10 mM of various cycloalkyl β-D-glucopyranosides. At regular intervals (1 min), 500 μL of 1 M Na_2CO_3 was added to the reaction mixture, and then the *p*-nitrophenol liberated was spectrophotometrically determined at 405 nm. The inhibition constants (K_i) for cycloalkyl β-D-glucopyranosides were calculated from Dixon and Webb plots,[20] and the type of inhibition was also reconfirmed by the method of Lineweaver-Burk.[21]

Preparation of cyclopentylmethyl β-glucopyranoside. Cellobiose (4.0 g) and CPEM (2.0 mL) were dissolved in 14.4 mL of 25 mM sodium acetate buffer (pH 5.0), and β-glucosidase (20 U) from *Trichoderma reesei* was added. After incubation for 72 h at 45°C, pH of the reaction mixture was adjusted to 3.0 with 1 N HCl, and then the reaction was terminated by heating for 10 min. The resulting insoluble materials were removed by centrifugation. The supernatant was loaded onto a Duolite S876 column (φ2.5×14 cm). The column was first washed with H_2O (210 mL) and then the desired product was eluted with 30% (w/w) methanol (140 mL). The eluate was concentrated, and a 1/5 volume of the concentrated was loaded on to a column (φ2.6×90 cm) of Toyopearl HW-40S (Toso Co., Japan). The column was eluted with distilled water at 65°C (flow rate; 30 mL/h). Elution was monitored by measurement at 485 nm. Total carbohydrate content in each fraction was determined by the phenol-sulfuric acid method. The eluate (each 10 mL fraction) gave a CPEM-β-G peak (tube Nos. 49–55) after the elution of the glucose peak (tube Nos. 16–19). The remaining aliquots were similarly worked up as above. The fractions corresponded to CPEM-β-G peak were combined, concentrated and lyophilized to obtain the purified CPEM-β-G in a final yield of 250 mg. NMR data (D_2O): ^{1}H, δ 4.45 (1H, d, J=7.92Hz, H-1β). ^{13}C-NMR data (D_2O): δ 105.23, 78.78, 78.72, 77.99, 76.03, 72.54, 63.65, 41.58, 31.88, 31.88, 27.78. The enzymatic syntheses of the other β-D-glucopyranosides were performed by the same procedures as in the case of CPEM-β-G, except for the difference of cyclic alcohols used as the aglycons. CPAM-β-G; NMR data (D_2O): ^{1}H, δ4.47 (1H, d, J=7.91Hz, H-1β). ^{13}C-NMR data (D_2O): δ104.41, 78.69, 78.42, 78.06, 75.96, 72.44, 63.57, 12.49, 5.44, 5.15. CPE-β-G; NMR data (D_2O): ^{1}H, δ4.48 (1H, d, J=7.91Hz, H-1β). ^{13}C-NMR data (D_2O): δ103.72, 85.17, 78.76, 78.71, 76.01, 72.58, 63.70, 35.36, 34.50, 25.76, 25.52. The chemical structures of the cycloalkyl β-glucopyranosides are shown in Fig. 1.

Preparation of crude flower enzyme extracts. Crude enzyme extracts from buds and blooming flowers were prepared by the method of Watanabe *et al*.[22] with slight modification as follows. Buds and blooming flowers (70 g) frozen by liquid nitrogen were homogenized in 500 mL of a cold acetone at −20°C by a homogenizer. They were filtrated, and the residues were washed with a cold acetone (500 mL) until the floral aromas were completely lost. Then the residues were dried *in vacuo* to prepare an acetone powder. Each 0.5 g acetone powder from *Lilium* cv. Le Reve and *Rosa hybrida* cv. Wendy was suspended in 200 mL of 0.1 M citrate buffer (pH 4.5) containing 1% Triton X-100. The suspensions were stirred at 4°C for 2 h, and insoluble materials were removed by centrifugation. Each supernatant was filtrated by a 0.22 μm membrane, and concentrated to 10 mL using an ultrafiltration membrane PM-10 (Amicon). The activities of β-glucosidases extracted from *Lilium* cv. Le Reve and *Rosa hybrida* cv. Wendy were 0.55 and 0.56 U/mL, respectively. These two concentrated flower extracts were used as crude enzymes for further kinetic analyses.

RESULTS AND DISCUSSIONS

Enzymatic syntheses of cycloalkyl β-glucopyranosides. On screening for β-glucosidases that produce a large amount of transglucosylation products from cyclic alcohols and cellobiose (Fig. 1), the yields of cycloalkyl β-D-glucopyranosides were dependent on both the chemical structure of the acceptor molecule and enzyme specificity as shown in Fig. 2. Interestingly, the enzymes from *A. ni-*

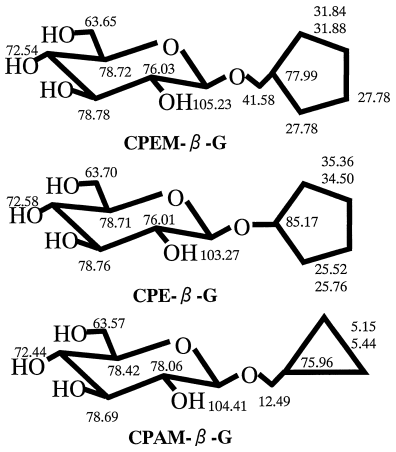

Fig. 1. Chemical structures of cycloalkyl β-D-glucopyranosides.

CPAM-β-G, CPE-β-G and CPEM-β-G indicate cyclopropylmethyl β-D-glucopyranoside, cyclopentyl β-D-glucopyranoside and cyclopentylmethyl β-D-glucopyranoside, respectively. Numbers indicate ^{13}C-NMR data (D_2O).

ger (Novozyme 188) and sweet almond (BGH-101) synthesized little/or no transglucosylation product compared with those from other fungi tested. On the basis of its high productivity of transglucosylation products, we selected the enzyme from *Trichoderma reesei* (Cellcrust). When CPAM, CPE and CPEM were used as acceptor substrates, the corresponding β-D-glucopyranosides were obtained in the yields of 8.0, 9.9 and 3.6% (HPLC area%) at 148 h reaction, respectively. The desired β-D-glucopyranosides contents gradually decreased if the reaction continued longer. That is, it is considered that the rates of hydrolysis and transglycosylation are evenly balanced at 148 h reaction. Although the chemical structures of CPE and CPEM were very similar to each other from the viewpoint that both cyclic alcohols have a cyclopentane ring, the yields of transglucosylation products were quite different (CPE: 9.9%, CPEM: 3.6%). On the other hand, the yields of the products synthesized enzymatically were at almost the same level although CPE and CPAM have quite a difference in their chemical structure. However, both cyclic alcohols have an alkyl carbon between the ring structure and the hemiacetal OH residue as shown in Fig. 1. These differences in the yields of transglucosylation products related to the chemical structure of aglycon indicate the possibility that the selective inhibitor could be designed by not only glycon residue but also aglycon structure.[16,23] The chromatographic separations of the desired compounds CPAM-, CPE- and CPEM-β-G were carried out using an adsorbed resin column, followed by a gel filtration column. Finally, these compounds were obtained in the yields of 12.7, 8.3 and 7.2% (mol%) on the basis of donors added, respectively.

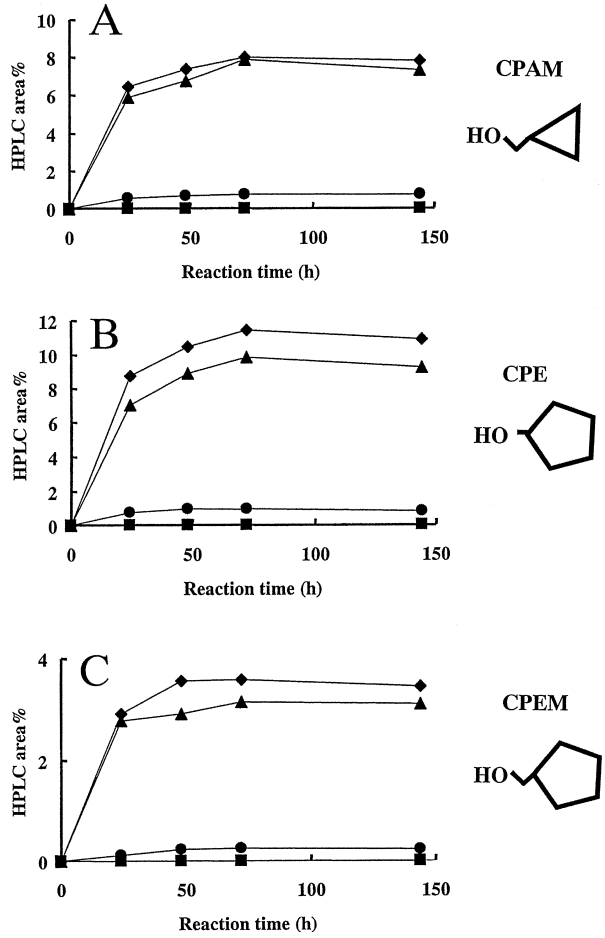

Fig. 2. Courses of the formation of transglucosylation products.

Cycloalkyl β-D-glucopyranosides were synthesized by transglucosylation reaction with β-glucosidases from *Trichoderma reesei* (◆), *A. niger* (●), *T. viride* (▲), and sweet almond (■), respectively. The donor substrate in all reactions was cellobiose and the acceptors were CPAM (A), CPE (B) and CPEM (C), respectively.

Recognition of glucose residue and cyclic alcohols by β-glucosidases.

Before the inhibition analyses for cycloalkyl β-D-glucopyranosides using β-glucosidases from *Trichoderma viride*, *Aspergillus niger* and sweet almond, both K_m values for pNP-β-G and K_i values for glucose from three purified β-glucosidases were estimated by Lineweaver-Burk plots. On the basis of the plots of $1/v$ against $1/S$, the kinetic parameters were determined by the Michaelis-Menten equation as summarized in Table 1. When glucose is regarded as an inhibitor, the K_i value for glucose yielded the enzyme from sweet almond (347 mM) is apparently larger than those by the enzymes from fungi. The inhibition type for glucose in the former plant enzyme was a mixed type; however the latter fungi enzymes were inhibited in a competitive manner (Table 1). As shown in Table 2, IC_{50} (concentration of the inhibitor required to cause 50% inhibition) values of cyclic alcohol and the corresponding β-D-glucopyranosides used were determined using pNP-β-G and the above three enzymes. Although all alcohols tested had no inhibitory activity for β-glucosidases from *A. niger* and *T. viride*, a relatively

Table 1. Kinetic parameters of β-glucosidases for pNP-β-G.

	K_m (mM)	k_o (s^{-1})	Glucose	
			K_i (mM)	Inhibition type
Almond	2.37±0.11	65.2±8	347±20	Mixed
Almond*[25]	2.24±0.11	—	406	Mixed
Almond*[26]	2.93±0.02	—	—	—
A. niger	0.61±0.02	172±8	1.52±0.12	Competitive
*A. niger**[27]	0.57±0.04	270±30	—	—
T. viride	0.11±0.01	68.2±5	0.34±0.02	Competitive
*T. viride**[28]	0.5	—	0.53	Mixed (competitive)

*For comparison, the data obtained for sweet almond,[25,26] *A. niger*[27] and *T. viride*[28] are also shown.

Table 2. Inhibitory activity of alcohols and their corresponding β-D-glucopyranosides.

	IC_{50} (mM)			
	CPAM	CPE	CPEM	Glucose
Almond	357	79.5	5.86	836
A. niger	NI*	NI*	NI*	6.6
T. viride	NI*	NI*	NI*	3.6
	CPAM-β-G	CPE-β-G	CPEM-β-G	
Almond	42.1	33.6	0.68	
A. niger	19.1	15.3	33.2	
T. viride	17.5	6.76	12.4	

*NI, no inhibitory activity was observed.

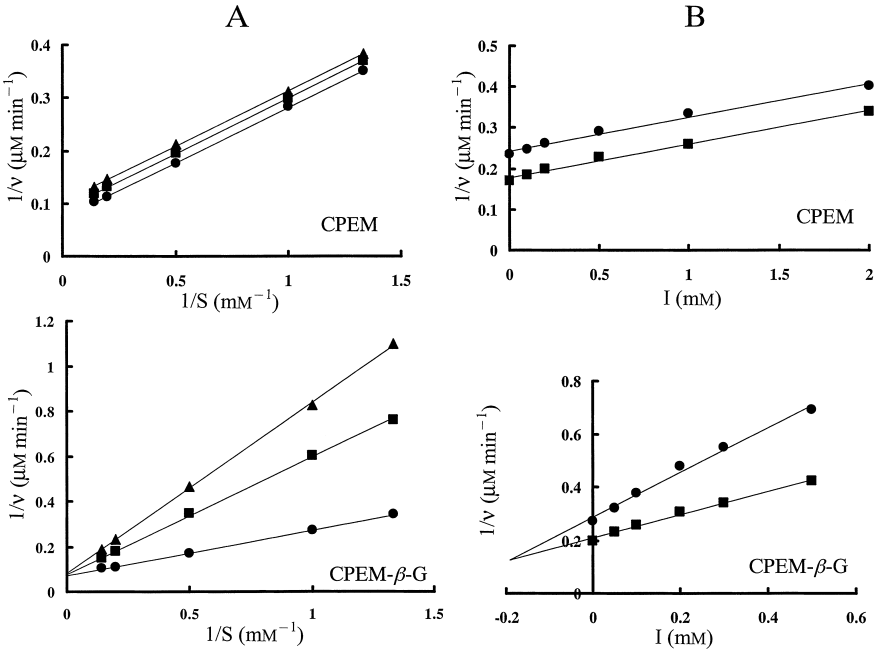

Fig. 3. Lineweaver-Burk (A) and Dixon plots (B) of sweet almond β-glucosidase reaction.

(A) The reciprocal of the specific activity ($1/v$) is plotted against the reciprocal of pNP-β-G concentrations at fixed CPEM or CPEM-β-G concentrations; 0.5 (▲), 0.25 (■) and 0 (●) mM. (B) The reciprocal of the specific activity ($1/v$) is plotted against the reciprocal of CPEM or CPEM-β-G concentrations at fixed pNP-β-G concentrations; 3 (●) and 6 (■) mM.

weak inhibitory activity for sweet almond enzyme was observed. On the contrary, most of the corresponding cycloalkyl β-D-glucopyranosides had various ranges of inhibitory activities for these three enzymes. CPEM-β-G was found to show the strongest inhibitory activity (IC_{50} $=0.68$ mM) for sweet almond β-glucosidase, and it could not be hydrolyzed under the enzyme assay conditions described in MATERIALS AND METHODS. Therefore, we attempted to determine the kinetic parameters to gain insights into the detailed action mechanism of CPEM and CPEM-β-G on the above three enzymes.

Kinetic parameters of the enzymes for CPEM and CPEM-β-G.

The effects of CPEM and CPEM-β-G on sweet almond β-glucosidase activity were examined using both Lineweaver-Burk and Dixon plots (Fig. 3), and the kinetic parameters for three β-glucosidases are summarized in Table 3. Among the enzymes tested, a β-glucosidase from sweet almond was the most strongly inhibited by CPEM-β-G ($K_i=0.15\pm0.02$ mM). Although CPEM itself had no inhibitory activity toward the enzymes from fungi, it had a weak inhibitory activity for sweet almond enzyme (K_i $=1.11\pm0.2$ mM). Interestingly, the inhibition type of CPEM was uncompetitive; however, it was changed to a competitive type by introducing a glucose molecule to the corresponding alcohol as shown in Fig. 3. The K_i value of CPEM-β-G for the enzyme fell to about $1/7$ compared with that of the corresponding cyclic alcohol (Table 3). These results suggest that sweet almond β-glucosidase is likely preferentially to use β-D-glucopyranosides possessing the hydrophobic molecule as an aglycon rather than starch or related α-(1,4)-linked glucose polymers which are easily utilized by fungi. The fact that plant aromatic substances such as monoterpene alcohols and aromatic alcohols exist generally in the form of β-D-glucopyranosides and that they are liberated by β-glucosidase digestion[24] seems to contribute to a better understanding of the present results mentioned above.

Table 3. K_i values for inhibitors of β-glucosidases using pNP-β-G as a substrate.

β-Glucosidase	K_i (mM) Inhibition type		
	Glucose	CPEM	CPEM-β-G
Almond	347±20	1.10±0.07	0.15±0.02
	Mixed	Uncompetitive	Competitive
A. niger	1.52±0.12	NI*	19.6±0.7
	Competitive		Mixed
T. viride	0.34±0.02	NI*	1.51±0.2
	Competitive		Competitive

*NI, no inhibitory activity was observed.

It is well known that a glucono-1,5-lactone, which has a structural similarity with the oxocarbonium ion-like transition state intermediates, is a strong inhibitor for β-glucosidases.[17–19] A glucono-1,5-lactone inhibits β-glucosidases independently of their origins and its inhibition type is a competitive one. CPEM-β-G prepared in this work also inhibits the sweet almond enzyme by the same inhibition type as is the case of glucono-1,5-lactone. However, its inhibition activity depends on the origin of the β-glucosidases. These facts indicate that the inhibition activity of CPEM-β-G is caused by the interaction between the enzyme and hydrophobic residue (CPEM), and a more specific inhibitor for plant enzymes would be obtained by screening a more suitable structure of aglycon.

Effects of CPEM-β-G on the activity of cut flower enzymes.

Generally, the moderate and marvelous fragrant breath of flowers holds the clues for relaxation; however, strong

Table 4. Effects of inhibitors on the activity of β-glucosidases extracted from the flowers.

	No inhibitor	CPEM	CPEM-β-G	CPAM-β-G	CPE-β-G
		5 mM			
Lilium cv. Le Reve	1.0	0.84*	0.46*	0.94*	0.8*
Rosa hybrida cv. Wendy	1.0	0.51*	0.24*	0.58*	0.54*
		10 mM			
Lilium cv. Le Reve	1.0	0.73*	0.28*	0.82*	0.61*
Rosa hybrida cv. Wendy	1.0	0.37*	0.16*	0.42*	0.54*

*Relative activities were expressed.

and/or unpleasant ones may need to be tempered for certain situations, for example, in the hospital. As it was projected that CPEM-β-G is much more likely to inhibit the enzyme activity from plant origin compared with those from fungi, we confirmed the possibility of CPEM-β-G inhibiting the activities of β-glucosidases from cut flowers on the basis of kinetic studies. Crude enzyme extracts were prepared from 70 g of flower buds (*Lilium* cv. Le Reve *and Rosa hybrida* cv. Wendy) by the method of Watanabe *et al.*,[22] and the effects of CPEM-β-G on the pNP-β-G hydrolyzing activities of crude flower enzymes were also investigated. Consequently, CPEM-β-G inhibited pNP-β-G hydrolyzing activity (inhibition rate of 54–84%) at the concentration of 5–10 mM in the reaction mixture as shown in Table 4.

Furthermore, we attempted to carry out preliminarily the following examinations by the organoleptic test. The cut ends of flower materials (*Lilium* cv. Le Reve) were immediately put in tap water, their lengths being adjusted to about 15 cm. To investigate the effect of CPEM-β-G on the formation of volatile compounds in cut flowers, each flower was placed in 5 mM CPEM-β-G solution (50 mL) or distilled water as a control in a 200 mL flask. They were kept at 23°C, 70% relative humidity and 1000 Lx irradiance using cool-white fluorescence lamps under a 12 h photoperiod (FLI-301NH incubator, Tokyo Rika Co., Ltd. Japan). After three days, the fragrant breath of flowers was examined by organoleptic test (10 expert panelists). The 8 panelists judged that the aroma was distinctly reduced by CPEM-β-G treatment. This result suggested that strong and/or unpleasant flowers fragrances could be reduced by use of β-glucosidase inhibitors. We are planning to analyze the mechanisms of reducing the unpleasant flower aroma using β-glucosidase inhibitors in future studies.

In conclusion, the process of the enzymatic preparation of the desired cycloalkyl β-D-glucopyranosides is rather simple and their yields are sufficiently high. CPEM-β-G is one of the novel inhibitors, which has a relatively simple chemical structure without a nitrogen atom in the position of the anomeric oxygen or the ring oxygen atom.[16] Studies on the enzyme inhibitors by modifying the substrate structures would be not only helpful in revealing the requirements for the binding and catalytic specificity of the enzymes but also expected to develop a novel practical use for them.

We wish to thank Dr. G. Okada, professor emeritus of Shizuoka University for his critical reading of the manuscript.

REFERENCES

1) Y. Someya, H. Matsui and S. Chiba: Chemical modification of histidyl residue in the active site of brewer's yeast α-glucosidase II. *Agric. Biol. Chem.*, **48**, 873–879 (1984).
2) B.Y. Tao, P.J. Reilly and J.F. Robyt: Detection of a covalent intermediate in the mechanism of action of porcine pancreatic α-amylase by using ^{13}C nuclear magnetic resonance. *Biochim. Biophys. Acta*, **995**, 214–220 (1989).
3) G. Mooser, S.A. Hefta, R.J. Paxton, J.E. Shively and T.D. Lee: Isolation and sequence of an active-site peptide containing a catalytic aspartic acid from two *Streptococcus sobrinus* α-glucosyltransferases. *J. Biol. Chem.*, **266**, 8916–8922 (1991).
4) Y. Isoda, Y. Shimizu, A. Hashimoto, H. Fujiwara, Y. Nitta and A. Kagemoto: Mechanism of hydrolyses of phenyl α-maltosides catalyzed by taka-amylase A. *J. Biochem.* (Tokyo), **111**, 204–209 (1992).
5) C.C. Blake, L.N. Johnson, G.A. Mair, A.C. North, D.C. Phillips and V.R. Sarma: Crystallographic studies of the activity of hen egg-white lysozyme. *Proc. R. Soc. Lond. B. Biol. Sci.*, **167**, 378–388 (1967).
6) L.O. Ford, L.N. Johnson, P.A. Machin, D.C. Phillips and R. Tjian: Crystal structure of a lysozyme-tetrasaccharide lactone complex. *J. Mol. Biol.*, **88**, 349–371 (1974).
7) H. Matsui, J.S. Blanchard, C.F. Brewer and E.J. Hehre: α-secondary tritium kinetic isotope effects for the hydrolysis of α-D-glucopyranosyl fluoride by exo-α-glucanases. *J. Biol. Chem.*, **264**, 8714–8716 (1989).
8) A. Cogoli and G. Semenza: A probable oxocarbonium ion in the reaction mechanism of small intestinal sucrase and isomaltase. *J. Biol. Chem.*, **250**, 7802–7809 (1975).
9) S. Horii, T. Iwasa and Y. Kameda: Studies on validamycins, new antibiotics. V. Degradation studies. *J. Antibiot*. (Tokyo), **24**, 57–58 (1971).
10) S. Horii, H. Fukase, T. Matsuo, Y. Kameda, N. Asano and K. Matsui: Synthesis and α-D-glucosidase inhibitory activity of N-substituted valiolamine derivatives as potential oral antidiabetic agents. *J. Med. Chem.*, **29**, 1038–1046 (1986).
11) D.D. Schmidt, W. Frommer, B. Junge, L. Muller, W. Wingender, E. Truscheit and D. Schafer: α-Glucosidase inhibitors. New complex oligosaccharides of microbial origin. *Naturwissenschaften*, **64**, 535–536 (1977).
12) S. Inouye, T. Tsuruoka, T. Ito and T. Niida: Structure and synthesis of nojirimycin. *Tetrahedron*, **24**, 2125–2144 (1968).
13) H. Paulsen: Kohlenhydrate mit sticstoff oder schwefel im "halbacetal" -ring. *Angew. Chem.*, **78**, 501–556 (1966).
14) B. Junge, H.P. Krause, L. Muller and W. Puls: Neue derivate von 3,4,5-trihydroxy-piperidin, verfahren zu ihrer herstellung und ihre verwendung. *Ger Offen* 2758025 (prior 24.12.77) (1979).
15) D.L. Zechel and S.G. Withers: Glycosyl transferase mechanisms. in *Comprehensive Natural Products Chemistry*, vol. 5, C.D. Poulter, ed., Pergamon, Amsterdam, 279–314 (1999).
16) W. Guo, J. Hiratake, K. Ogawa, M. Yamamoto, S. J. Ma and K. Sakata: β-D-Glycosylamidines: Potent, selective, and easily accessible β-glycosidase inhibitors. *Bioorg. Med. Chem. Lett.*, **11**, 467–470 (2001).

17) G.A. Levvy, A.J. Hay and J. Conchie: Inhibition of glycosidases by aldonolactones of corresponding configuration. 4. Inhibitors of mannosidase and glucosidase. *Biochem. J.*, **91**, 378−384 (1964).
18) M. Takada, K. Ogawa, S. Saito, T. Murata and T. Usui: Chemo-enzymatic synthesis of galactosyl maltooligosaccharidono-lactone as a substrate analogue inhibitor for mammalian α-amylase. *J. Biochem.*, **123**, 508−515 (1998).
19) M. Takada, K. Ogawa, T. Murata and T. Usui: Inhibition of human α-amylases by synthetic 6^3-*O*-β-D-galactosyl maltobionolactones. *J. Carbohydr. Chem.*, **18**, 149−163, (1999).
20) M. Dixon and E.C. Webb: The Enzymes, 2nd Ed. Longmans, Essex (1964).
21) H. Lineweaver and D. Burk: The determination of enzyme dissociation constants. *J. Am. Chem. Soc.*, **56**, 658−666 (1934).
22) N. Oka, H. Ohishi, T. Hatano, M. Hornberger, K. Sakata and N. Watanabe: Aroma evolution during flower opening in *Rosa damascena* mill. *Z. Naturforsch.*, **54**, 889−895 (1999).
23) K. Inoue, J. Hiratake, M. Mizutani, M. Takada, M. Yamamoto and K. Sakata: β-glycosylamidine as ligand for affinity chromatography tailored to the glycon substrate specificity of β-glycosidases. *Carbohydr. Res.*, **338**, 1477−1490 (2003).
24) S.J. Ma, M. Mizutani, J. Hiratake, K. Hayashi, K. Yagi, N. Watanabe and K. Sakata: Substrate specificity of β-primeverosidase, a key enzyme in aroma formation during oolong tea and black tea manufacturing. *Biosci. Biotechnol. Biochem.*, **65**, 2719−2729 (2001).
25) N. Baggett, M.A. Case, P.R. Darby and C.J. Gray: Action of almond β-D-glucosidase on fluorogenic substrates derived from 4-substituted 7-hydroxycoumarins. *Enzyme Microb. Technol.*, **15**, 742−747 (1993).
26) A. Romeu, M.A. Montero, J.A. Ruiz, M.J. De Maria, J.A. Jimenez, A. Rojas and L. Arola: Effects of glucose, ethanol, Hg(II) and Cu(II) on almond β-glucosidase. *Biochem. Mol. Biol. Int.*, **33**, 939−946 (1994).
27) M. Ohnishi, G. Okada and T. Yazaki: Characterization of the subsite structure of the β-glucosidase from *Aspergillus niger*, an aspect of the mechanism of carbohydrate recognition. *Carbohydr. Res.*, **308**, 201−205 (1998).
28) M.A. Montero and A. Romeu: Kinetic study on the β-glucosidase-catalysed reaction of *Trichoderma viride* cellulase. *Appl. Microbiol. Biotechnol.*, **38**, 350−353 (1992).

環状アルキル β-D-グルコピラノシドの酵素合成およびその植物起源 β-グルコシダーゼに対する阻害活性

高田正保，小川浩一
日本食品化工株式会社研究所
（417−8530 富士市田島 30）

Trichoderma reesei 起源の β-グルコシダーゼを用いて，β-グルコシダーゼ基質アナログ阻害剤として環状アルキル β-D-グルコピラノシドの酵素合成法を検討した．環状アルコールであるシクロプロパンメタノール（CPAM），シクロペンタノール（CPE）およびシクロペンタンメタノール（CPEM）を受容体基質に，セロビオースを供与体基質に用いた場合，アノマー選択的にそれぞれの環状アルコールに対応する環状アルキル β-D-グルコピラノシドの合成が可能であった．吸着樹脂およびゲルろ過により，合成された環状アルキル β-D-グルコピラノシドを精製後，*Trichoderma viride*，*Aspergillus niger* および sweet almond 起源の精製 β-グルコシダーゼに対する阻害効果を検討した．合成環状アルキル β-D-グルコピラノシドの中で，シクロペンチルメチル β-D-グルコピラノシド（CPEM-β-G）のみが sweet almond 起源の β-グルコシダーゼを拮抗的に阻害し（K_i＝0.15±0.02 mM），その他の環状アルキル β-D-グルコピラノシドは検討した β-グルコシダーゼに対してはほとんど阻害効果を示さなかった．CPEM 自体は sweet almond 起源の β-グルコシダーゼ活性を非拮抗的に阻害した．一方，本環状アルコールにグルコースを導入した CPEM-β-G の当該酵素に対する K_i 値は約 1/7 に減少し，かつ阻害形式は拮抗型を示した．一連の阻害実験から，CPEM-β-G は菌類起源よりむしろ植物起源の β-グルコシダーゼ活性を阻害しやすいという知見がみられた．実際，今回合成した配糖体のユリ切り花由来の酵素活性に対する影響を検討したところ，CPEM-β-G（5−10 mM）が最も強く阻害した（54−84%）．