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Glycosidase Inhibition by Cyclic Sulfonium Compounds

Hideya Yuasa,* Jun Takada and Hironobu Hashimoto

Department of Life Science, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 226-8501, Japan

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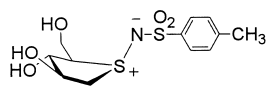
Abstract—Inhibitory activities of various cyclic sulfonium compounds including salacinol against several glycosidases were studied and some compounds showed significant inhibition. The sulfonium ion structure was found to be essential for the inhibitory activity. Specific inhibition of salacinol toward rice α-glucosidase was ascribed to the tether arm. © 2001 Elsevier Science Ltd. All rights reserved.

Carbohydrate-based cyclic sulfonium compounds are gaining a reputation as a new class of glycosidase inhibitor.[1–5] This class of inhibitor bases its activity on the sulfur atom, unlike most of the other glycosidase inhibitors that exert their activity on the basis of the nitrogen atom.[6,7] The sulfimide derivative **1**, a β-glycosidase inhibitor developed by Yuasa and co-workers,[2] is a forerunner of this class. This compound was designed to mimic the transition state structure **2**, which has been speculated for the hydrolysis of β-glucosides. Thereafter, salacinol **3** was discovered from the antidiabetic traditional Ayurvedic medicine,[3] and was found to have an extraordinarily strong inhibitory activity toward α-glucosidases. Salacinol is a cyclic sulfonium compound having a 1,4-epithio-D-arabinitol skeleton as is compound **1**. This research sought the behavior of various sulfonium derivatives of 1,4-epithio-D-arabinitol (**9**) toward various glycosidases, in order to demonstrate that the sulfonium-ion structure is actually related to its inhibitory activity and deserves further exploitation. Authors have previously reported the syntheses of salacinol **3**, its stereoisomer **4**, and the analogue (**5**) of compound **4** that replaced 1,4-epithio-D-arabinitol by tetrahydrothiophene **6**.[5] In this paper, the salacinol-type enantiomer (**8**) of the above-mentioned analogue **5** and the methyl sulfonium derivative (**10**) of 1,4-epithio-D-arabinitol were newly synthesized.



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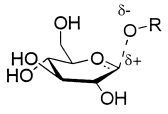

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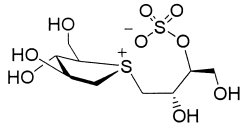

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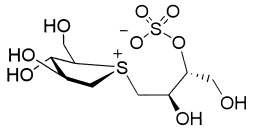

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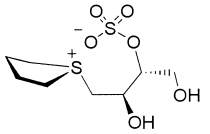

5

The synthesis of compound **8** followed that of compound **5** (eq 1). 2,4-*O*-Sulfonyl-1,3-*O*-isopropylidene-D-erythritol (**7**) was reacted with compound **6** via a concomitant ring-opening of the cyclic sulfate to give a coupled compound, which was then deisopropylidenated to give compound **8**.[8]

Compound **10** was synthesized by treatment of 1,4-epithio-D-arabinitol (**9**) with methyl iodide and $NaHCO_3$ (eq 2), and was obtained as a mixture (**10a**,**b**) of two stereoisomers, even HPLC (C18 column and CH_3CN/H_2O 0:100–30:70 gradient during 30 min) being unable to separate them. However, when silica gel chromatography was used, the stereoisomer ratios of the first

*Corresponding author. Tel.: +81-45-924-5705; fax: +81-924-5805; e-mail: hyuasa@bio.titech.ac.jp

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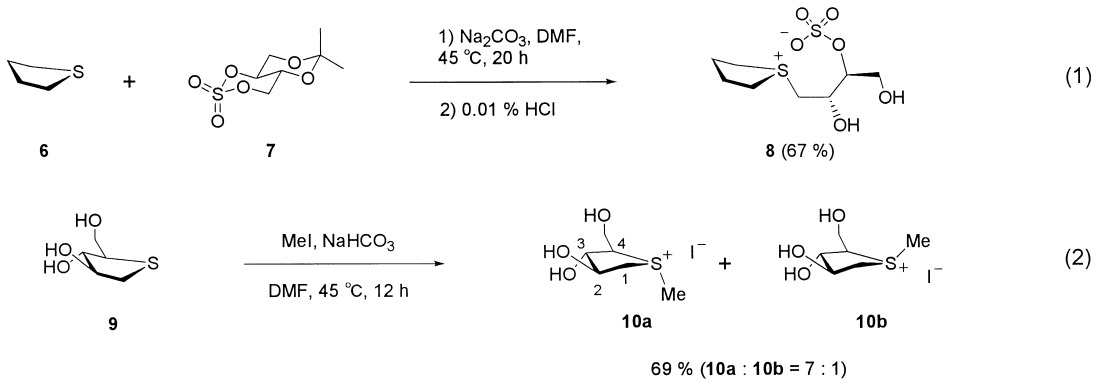

Table 1. IC_{50} (mM) of cyclic sulfonium compounds toward various glycosidases[a]

Compound	α-Glcase[b]				β-Glcase[c]	α-Manase[d]
	Rice	Baker's yeast	*S. cerevisiae* (recombinant)	*B. stearothermophilus*	Almond	Almond
3	1.1×10^{-3}	na[e]	na	2.5	na	2.1[f]
4	0.38	na	na	na	3.4	3.6
5	1.0	na	na	nd	nd	nd
8	na	na	nd	nd	nd	nd
10a/10b = 10:1	0.41	0.36	0.49	0.25	na	2.1
10a/10b = 4:6	2.0	2.2	2.1	1.4	na	1.2

[a]All enzyme reactions were performed in 22.5 mM citrate buffer (450 μL) containing a *p*-nitrophenyl glycopyranoside, enzyme, and an inhibitor for 10 min and stopped by adding 0.9 mL of 50 mM glycine buffer (pH 10). Concentration of the liberated *p*-nitrophenol was determined by absorbance at 400 nm.
[b]α-Glucosidase: *p*-nitrophenyl α-D-glucopyranoside was used as a substrate at 37 °C (pH 6.8).
[c]β-Glucosidase: *p*-nitrophenyl β-D-glucopyranoside was used as a substrate at 25 °C (pH 5.5).
[d]α-Mannosidase: *p*-nitrophenyl α-D-mannopyranoside was used as a substrate at 25 °C (pH 4.5).
[e]Not active (less than 50% inhibition) at 5 mM.
[f]40% inhibition.
[g]Not determined.

fraction and the last fraction were different from each other, with the mixing ratio (**10a**/**10b**) of 10:1 and 4:6, respectively, as determined by ^{1}H NMR.[9] The stereochemistry of the ring sulfur atom was determined by the position of the protons that have a strong NOE correlation with the methyl substituent. It was H-2 for compound **10a** and H-3 for compound **10b**. *J*-Values in ^{1}H NMR supported the envelope conformation (^1E) for both compounds **10a** and **10b**. This compound was inert to 0.1% HCl and 0.1% NaOH at room temperature for at least 24 h. However, it was fully decomposed within 1 h in 1 M NaOH and ca. 30% decomposed in 1% HCl overnight. Inhibition activities were investigated for various kinds of glycosidases with regard to salacinol, its derivatives synthesized previously, and the cyclic sulfonium compounds synthesized in this study. The inhibitory activity (IC_{50}) was determined for four α-glucosidases, a β-glucosidase, and an α-mannosidase, all of which were commercially available (Table 1). All inhibitors examined had no inhibitory effects at more than 2 mM toward the α-galactosidase from green coffee beans and the β-galactosidase from *E. coli* at 25 °C (pH 6.5). Salacinol (**3**) showed a strong inhibitory effect toward the rice-derived α-glucosidase, but a weak or no inhibition toward the other glycosidases. This result suggests a relevance to the fact that the rice α-glucosidase has a high substrate specificity to maltose and the others barely recognize the aglyconic structure of disaccharides. In practice, the α-glycosidases that were reported to be inhibited strongly by salacinol have a high specificity for maltose, isomaltose, and sucrose. The inhibition activity toward the rice α-glucosidase is substantially decreased for the stereoisomer of salacinol **4**, indicating that the tether structure of salacinol is very important for the occurrence of its specific inhibition. The salacinol derivative **8** having no hydroxymethyl and hydroxyl groups and its enantiomer **5** showed further decreases in their inhibition activities. The tetrahydrothiophene ring of salacinol is regarded as a mimic of the glucose ring and it is reasonable that removal of the hydroxyl groups caused the sizable activity losses. The methyl sulfonium derivative **10** showed relatively strong inhibitory activities against the four α-glucosidases and an α-mannosidase. The mixture of high *R*-isomer (**10a**) content showed the stronger inhibition than the equivalent mixture. It is noteworthy that the inhibitory activities of the methyl sulfonium derivative are mostly identical regardless of the origin of the glycosidases. Since 1,4-epithio-D-arabinitol (**9**) had no inhibitory activity toward all the glycosidases examined, even at 12 mM, the origin of the inhibitory activity of the methyl sulfonium **10a** is in its sulfonium ion structure.

In conclusion, we found the following: (1) sulfonium ion is an essential structure for the inhibitory activity of cyclic sulfonium compounds; (2) salacinol is a specific

inhibitor for the α-glucosidases designated to the hydrolysis of maltose; and (3) the specificity is generated by the tether arm. These findings indicate a possibility that the sulfonium ion structure would conserve a common inhibitory mechanism for all glycosidases and an appropriate adjustment of the tether structure would lead to discovery of a novel glycosidase inhibitor of strong activity and high specificity.

Acknowledgements

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8. Compound **8**: $[\alpha]_D^{23}$ +21.7° (*c* 1.2, methanol); ^{1}H NMR (400 MHz, D_2O) δ 4.41–4.34 (m, 2H, H-2,3), 3.98 (dd, *J* = 2.9, 12.8 Hz, 1H, H-1), 3.88 (dd, *J* = 3.1, 12.8 Hz, 1H, H-1′), 3.71–3.50 (m, 6H, H-4, H-4′, $CH_2CH_2S\times2$), 2.42–2.23 (m, 4H, $CH_2CH_2S\times2$); ^{13}C NMR (67.8 MHz, D_2O) δ 79.9, 65.4, 59.5, 46.9, 44.9, 44.4, 28.3, 28.2; HRMS (ESI) calcd for $[C_8H_{16}O_6S_2+H]^+$ 273.0475, found 273.0472.
9. Compound **10**: $[\alpha]_D^{23}$ −3.5° (*c* 0.61, H_2O) for 10:1 (**10a**/**10b**) mixture, $[\alpha]_D^{23}$ +9.8° (*c* 1.1, H_2O) for 4:6 (**10a**/**10b**) mixture; ^{1}H NMR (400 MHz, D_2O at 45 °C) for 4:6 (**10a**/**10b**) mixture δ 4.90 (dt, *J* = 4.12, 4.0, 4.0 Hz, 0.4H, H-2a), 4.56 (dd, 0.4H, H-3a), 4.51 (dt, *J* = 4.3, 4.2, 4.3 Hz, 0.6H, H-2b), 4.38 (dd, *J* = 4.2, 4.2 Hz, 0.6H, H-3b), 4.26 (d, *J* = 7.9 Hz, 0.4H, H-5a), 4.18 (dd, *J* = 4.3, 11.5 Hz, 0.4H, H-5b), 4.10 (dd, *J* = 3.0, 8.4 Hz, 0.4H, H-4a), 4.08–3.97 (m, 1.2H, H-4b, H-5b), 4.07 (t, *J* = 7.9, 7.9 Hz, 0.4H, H-5a′), 3.99 (dd, *J* = 3.8, 13.3 Hz, 0.4H, H-1a), 3.90–3.80 (m, 0.6H, H-1b), 3.88 (dd, *J* = 4.4, 13.3 Hz, 0.4H, H-1a′), 3.32 (dd, *J* = 4.2, 14.1 Hz, 0.6H, H-1b′); ^{13}C NMR (67.8 MHz, D_2O) δ 77.8, 77.7, 77.4, 76.9, 70.5, 70.3, 59.2, 57.2, 47.8, 45.9, 27.9, 21.7; HRMS (ESI) calcd for $[C_6H_{13}O_3S]^+$ 165.0585, found 165.0577. The existence of iodide ion was confirmed by AgI precipitation of pale-yellow color with $AgNO_3$ addition and by I_2 formation with $NaIO_4$ addition as evidenced by the clear to purple color change of the solution. Although the elemental analysis supported the existence of iodide ion, experimental error was very large as usual in the elemental analyses of the compounds having both sulfur and halogen atoms.