Synthesis and Evaluation of Gallotannin Derivatives as Antioxidants and α-Glucosidase Inhibitors

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Gallotannins are phenolic natural products containing galloyl moieties connected to polyhydric alcohol cores, e.g., D-glucose. Some gallotannins are reported to have antidiabetic properties, such as α-glucosidase inhibitory activity. In this study, fourteen unnatural gallotannin derivatives with 1,5-anhydroalditol and inositol as the cyclic polyol cores were synthesized to investigate how their structures affected antioxidative and α-glucosidase inhibitory activities. Tannic acid demonstrated the most potent antioxidative activity (EC\textsubscript{50} = 2.84 μM), with potency increasing proportionally to the number of galloyl moieties. Synthetic inositol derivatives outperformed 1,5-anhydroalditol derivatives in rat α-glucosidase inhibitory activity. Pentagalloyl glucose, a natural compound, demonstrated the highest activity (IC\textsubscript{50} = 0.336 μM).

Key words gallotannin; maplexin; α-glucosidase; 1,5-anhydro-D-glucitol; antioxidant

Introduction

Gallotannins are phenolic natural products in the hydrolyzable tannin class with diverse structures and wide distribution in the plant kingdom. One characteristic structural feature of the gallotannins is that one or more gallic acid(s) is/are condensed to a polyol core such as α-glucose. For example, penta-galloylated glucose (PGG),\textsuperscript{3} tannic acid,\textsuperscript{2} acertannin,\textsuperscript{3} pycnalin\textsuperscript{4} and scyllo-querцитol gallate\textsuperscript{5} are known as plant secondary metabolites (Fig. 1).

We recently focused on 1,5-anhydro-D-glucitol (1,5-AG; 1, Chart 1) as the core structure of a series of gallotannin derivatives due to its antidiabetic properties and systematically synthesized analogs to evaluate structure–activity relationships (α-glucosidase inhibition and antioxidant).\textsuperscript{6} Condensing galloyl units on the core was found to influence the α-glucosidase inhibitory activity, and increasing the number of galloyl units significantly increased the inhibitory activity. The fully galloylated compound, 2,3,4,6-tetra-O-galloyl-1,5-AG\textsuperscript{7} (maplexin J; 2, Chart 1) demonstrated significant activities. Based on these results, the present report describes the synthesis and evaluation of the biological activities of novel gallotannins with different core configurations. Additionally, several kinds of inositol derivatives having six galloyl units were prepared to investigate the effect of increasing the number of galloyl units against α-glucosidase inhibitory and antioxidant activity.

Experimental

Chemical Reagents Derivatives employing 1,5-anhydroalditol (Chart 1) as the core, including 1,5-AG, 1,5-anhydro-L-glucitol (ent-1,5-AG; 3), 1,5-anhydro-D-mannitol (1,5-AMan; 4), 1,5-anhydro-D-galactitol (1,5-AGal; 5), 1,5-anhydro-D-rhamnitol (1,5-ARha; 6), 1,5-anhydro-D-fucitol (1,5-AFuc; 7), and 1,5-anhydroxytol (1,5-AXyl; 8), were synthesized from a simple procedure using glycosyl iodide according to our previous report.\textsuperscript{9} Inositols, including allo-, D-chiro-, L-chiro-, epi-, scyllo-, muco-, and myo-inositol, were commercially available.

Preparation of Galloylated 1,5-Anhydroalditol Analogs Several cyclic polyols were esterified with gallic acid derivative 9\textsuperscript{9} using 2-chloro-1-methylpyridinium iodide (2-CMPI) in the presence of a base to obtain benzyl-protected analogs in reasonable yields. Following deprotection of the benzyl ether under hydrogenolysis by Pd(OH)\textsubscript{2} on active carbon, several gallotannin derivatives 2, 10–22 were obtained in 51–98% total yields (Chart 1, see Supplementary Materials). All the synthesized galloylated derivatives are novel compounds, except for 1,5-AG derivative 2 and per-O-galloyl-myoinositol 22.\textsuperscript{10}

Antioxidant and α-Glucosidase Inhibitory Activity Assays The antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity and trolox-equivalent using a 96-well microplate based on previ-
Table 1. Results of Antioxidant and α-Glucosidase Inhibition (α-GI) Assays

| Compounds | Antioxidant | α-GI (Yeast) | α-GI (Rat) |
|-----------|-------------|--------------|------------|
| 1,5-AG (2) | EC_{50} (μM) 6.81 ± 0.17 | Trolox-eq. IC_{50} (μM) 1.06 ± 0.04 | | |
| ent-1,5-AG (10) | 7.12 ± 0.80 | 1.17 ± 0.15 | 2.30 ± 0.10 |
| 1,5-AMan (11) | 6.99 ± 0.06 | 7.57 ± 0.45 | 5.19 ± 1.21 |
| 1,5-AGal (12) | 7.16 ± 0.10 | 7.01 ± 0.57 | 2.88 ± 0.28 |
| 1,5-ARha (13) | 0.10 ± 0.56 | 5.88 ± 0.31 | 1.38 ± 0.28 |
| 1,5-AFuc (14) | 9.88 ± 0.04 | 4.96 ± 0.30 | 20.3 ± 0.5 |
| 1,5-AXyl (15) | 10.0 ± 0.4 | 4.93 ± 0.54 | 4.89 ± 0.29 |
| PGG | 5.90 ± 0.51 | 8.07 ± 0.44 | 0.211 ± 0.033 | 0.336 ± 0.040 |
| allo (16) | 5.12 ± 0.15 | 9.53 ± 1.03 | 0.166 ± 0.006 | 0.811 ± 0.178 |
| v-chiro (17) | 5.37 ± 0.43 | 8.09 ± 0.04 | 0.137 ± 0.003 | 0.659 ± 0.216 |
| t-chiro (18) | 5.12 ± 0.54 | 8.91 ± 0.54 | 0.137 ± 0.002 | 1.75 ± 0.09 |
| epi (19) | 6.00 ± 0.49 | 7.22 ± 0.16 | 0.149 ± 0.003 | 1.20 ± 0.21 |
| scyllo (20) | 4.24 ± 0.27 | 10.1 ± 0.4 | 0.200 ± 0.011 | 2.82 ± 0.43 |
| muco (21) | 5.46 ± 0.19 | 8.89 ± 0.92 | 0.121 ± 0.001 | 0.921 ± 0.045 |
| myo (22) | 6.80 ± 0.82 | 7.20 ± 0.83 | 0.110 ± 0.016 | 1.20 ± 0.03 |
| Tannic acid | 2.84 ± 0.05 | 16.2 ± 1.7 | 0.183 ± 0.013 | 0.866 ± 0.176 |
| Acarbose | — | — | 312.1 ± 9.8 | 0.113 ± 0.030 |
| 1,5-AG (2) | — | — | 1.53 ± 0.04 | 2.74 ± 0.08 |
| Acarbose | — | — | 455.2 ± 3.9 | 0.163 ± 0.010 |
| Trolox | 51.08 ± 2.26 | — | — | — |

a) Polyol core was shown for the synthesized derivative. b) EC_{50} and trolox-eq. represents mean ± standard deviation (S.D.) of n = 3. c) IC_{50} represents mean ± S.D. of n = 3. d) IC_{50} represents mean ± S.D. of n = 2. e) Dissolved in 10% DMSO due to low solubility in water. (2.5% final DMSO concentration).
ous reports. The yeast α-glucosidase inhibitory activity was determined using the p-nitrophenyl glucoside (PNPG) method on a 96-well microplate, according to a previous report with minor modification. The inhibitory activity of rat intestinal α-glucosidase was measured using a commercially available kit (FUJIFILM α-glucosidase inhibitory activity assay kit). The detailed protocols for these assays are described in the cited literature or supplementary materials.

Results and Discussion
Antioxidative and α-glucosidases (yeast and rat intestinal) inhibitory activities of all synthesized compounds were evaluated and compared to PGG, acarbose, tannic acid, or Trolox (Table 1).

Antioxidant activity was measured by the DPPH free radical scavenging assay and evaluated using the values of EC_{50} and trolox-equivalent (TE). Comparing the antioxidant activities of the tetra-galloyl derivatives, 2, 10–12, they showed almost the same values of EC_{50} and TE (EC_{50} = 6.81–7.16 μM, TE = 5.62–7.57). Similarly, comparing the antioxidant activities of tri-galloyl derivatives 13–15, they also showed almost the same values (EC_{50} = 9.06–10.0 μM, TE = 4.93–5.88) but weaker activities than the tetra-galloyl derivatives. PGG demonstrated a higher antioxidant activity (EC_{50} = 5.90 μM, TE = 8.07) than tri- and tetra-galloylated derivatives 2, 10–15. Hexa-galloyl containing inositol derivatives 16–18, 20, 21 had higher antioxidant activities (EC_{50} = 4.24–5.46 μM, TE = 8.09–10.1) than epip- and myo-inositol derivatives 19 and 22 (EC_{50} = 6.00, 6.80 μM, TE = 7.22, 7.20, respectively). Tannic acid, which has deca-galloyl moieties on the D-glucose core, displayed the highest antioxidant activity (EC_{50} = 2.84 μM, TE = 16.2). Regardless of the core structures, these results show that antioxidant activity is proportional to the number of galloyl units.

Comparing the inhibitory activity of yeast α-glucosidase, there was no difference between 1,5-AG derivative 2 and its enantiomer 10 (IC_{50} = 1.06, 1.17 μM, respectively). 1,5-AGal derivative 12 demonstrated slightly weaker activity against yeast α-glucosidase inhibition (IC_{50} = 1.67 μM) than 2 and 10, whereas 1,5-AMan derivative 11 demonstrated nearly twice the activity (IC_{50} = 0.626 μM) compared to 2, 10, and 12. The potency of the yeast α-glucosidase inhibitory activity of tri-galloyl derivatives 13–15 was lower (IC_{50} = 4.06–7.20 μM) than that of tetra-galloyl derivatives 2, 10–12. PGG exhibited stronger inhibitory activity against yeast α-glucosidase (IC_{50} = 0.211 μM) than those with fewer galloyl units, but inositol derivatives 16–22 and tannic acid performed even better (IC_{50} = 0.110–0.200 μM). The enantiomeric relationship between chiro-inositol derivatives 17 and 18 showed the same level of yeast α-glucosidase inhibitory activity (IC_{50} = 0.137 μM), similar to the enantiomers of 1,5-anhydroglucitol derivatives 2 and 10. In the comparison of the yeast α-glucosidase inhibitory activity, the myo-inositol derivative 22 demonstrated the highest inhibitory activity (IC_{50} = 0.101 μM). These results are comparable to the trend in antioxidant activity. The activity was less affected by the stereo configuration of galloyl moieties on the core but increased in proportion to the number of galloyl moieties.

On the other hand, comparing the inhibitory activities of rat intestinal α-glucosidase, 1,5-AG derivative 2, its enantiomer 10, and 1,5-AGal derivative 12 showed similar inhibitory activities (IC_{50} = 2.59–2.88 μM). However, 1,5-AMan derivative 11 showed lower inhibitory activity (IC_{50} = 5.19 μM). In the case of 1,5-ARha 13 and 1,5-AFuc derivative 14, which are 6-deoxy-sugar analogs, the activity relationship was reversed in rat intestinal α-glucosidase (IC_{50} = 4.53, 20.3 μM, respectively) compared with yeast α-glucosidase. Both 1,5-ARha derivative 13 and 1,5-AXyl derivative 15 having tri-galloyl units on the core showed similar inhibitory activity as 1,5-AMan derivative 11 containing tetra-galloyl units against rat intestinal α-glucosidase inhibitory activity. With regard to the rat intestinal α-glucosidase inhibitory activity of inositol derivatives, α-chiro-derivative 17 demonstrated the highest activity (IC_{50} = 0.659 μM). In contrast, ε-chiro-derivative 18 was lower (IC_{50} = 1.75 μM) than the other inositol derivatives 16, 19–22 (IC_{50} = 0.811–1.20 μM), with the exception of scyllo-derivative 20 dissolved in dimethyl sulfoxide (DMSO) (IC_{50} = 2.82 μM). Notably, PGG with “five” galloyl units demonstrated the highest inhibitory activity (IC_{50} = 0.336 μM) against rat intestinal α-glucosidase of all employed compounds, which was even higher than tannic acid (IC_{50} = 0.866 μM) with “ten” galloyl units.

Conclusion
We synthesized fourteen gallotannin derivatives, including twelve novel compounds, and evaluated their antioxidant and α-glucosidase inhibitor potentials. The antioxidant activity increased in direct proportion to the number of galloyl units. The yeast α-glucosidase inhibitory activity was also related to the number of galloyl units. However, the configuration of galloyl units also affected the activity of rat intestinal α-glucosidase, indicating a complex relationship between structure and inhibitory activity. Finally, it was revealed that PGG has the highest inhibitory activity against rat intestinal α-glucosidase among all the compounds investigated in this study.

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Conflict of Interest
The authors declare no conflict of interest.

Supplementary Materials
The online version of this article contains supplementary materials.

References
1) Torres-León C., Ventura-Sobrevilla J., Serna-Cock L., Ascacio-Valdés J. A., Contreras-Esquivel J., Aguilar C. N., J. Funct. Foods, 37, 176–189 (2017).
2) Chung K. T., Wong T. Y., Wei C. I., Huang Y. W., Lin Y., Crit. Rev. Food Sci. Nutr., 38, 421–464 (1998).
3) Bock K., Faureschau laCour N., Jensen S. R., Nielsen B. J., Phytochemistry, 19, 2033 (1980).
4) Ogawa A., Miyamae Y., Honma A., Koyama T., Yazawa K., Shimomori H., Chem. Pharm. Bull., 59, 672–675 (2011).
5) Nishimura H., Nonaka G.-I., Nishioka I., Phytochemistry, 25, 2599–
6) Machida S., Mukai S., Kono R., Funato M., Saito H., Uchiyama T., *Molecules*, **24**, 4340 (2019).

7) Ma H., Wang L., Niesen D. B., Cai A., Cho B. P., Tan W., Gu Q., Xu J., Seeram N. P., *RSC Adv.*, **5**, 107904–107915 (2015).

8) Uchiyama T., Shishikura K., Ogawa K., Ohshima Y., Miyairi S., *Tetrahedron Lett.*, **57**, 5294–5296 (2016).

9) Ren Y., Himmeldirk K., Chen X., *J. Med. Chem.*, **49**, 2829–2837 (2006).

10) Feldman K. S., Sambandam A., Lemon S. T., Nicewonger R. B., Long G. S., Battaglia D. F., Ensel S. M., Laci M. A., *Phytochemistry*, **51**, 867–872 (1999).

11) Yuan T., Wan C., Ma H., Seeram N. P., *Planta Med.*, **79**, 1674–1679 (2013).

12) Yokozawa T., Chen C. P., Dong E., Tanaka T., Nonaka G. I., Nishio I., *Biochem. Pharmacol.*, **56**, 213–222 (1998).