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Bio-inspired synthesis and bioactivities of phenylethanoid glycoside crenatosides†

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Herein, we report an effective approach for the synthesis of phenylethanoid glycosides bearing a fused 1,4-dioxane motif, employing a bio-inspired oxidative cyclization of the glucose 2′-OH and benzylic carbon of phenylethanol aglycone as a key step. Thus, crenatoside (**1**), isocrenatoside (**2**), and 20 analogs with varied substitution on the peripheral phenyl rings are synthesized. Some of the synthetic glycosides show considerable anti-inflammatory and immunosuppressive activities, and compound **36** exhibited the most potent immunosuppressive activity, with an IC_{50} value of 19.9 μM on B lymphocyte proliferation responses.

Introduction

Phenylethanoid (or phenylpropanoid) glycosides (PhGs) are a distinct class of natural products widely distributed in folk medicinal plants, particularly those belonging to the dicotyledonous group.[1] Structurally, PhGs are characterized by cinnamate and hydroxyphenylethyl moieties attached to D-glucopyranose through ester and β-glycosidic linkages, respectively.[1] To date, more than 500 PhGs have been isolated and characterized, which exhibit a wide range of pharmacological activities, such as antibacterial, antitumor, antiviral, anti-inflammatory, antioxidant, neuroprotective, and cardioprotective effects.[2] Being a prototypical PhG, acteoside was first extracted from dicotyledonous plants in 1963 (Fig. 1a); five years later, its structure was determined to be β-(3,4-dihydroxyphenyl)ethyl α-L-rhamnopyranosyl (1″→3′)-β-D-(4′-*O*-caffeoyl)glucopyranoside.[3] Two decades later, β-hydroxyacteoside (namely campneoside II), an oxidized derivative of acteoside, was isolated from a traditional Chinese medicinal plant *Forsythiae fructus*.[4] Having similar structural characteristics to campneoside II, crenatoside (**1**) was extracted from the aerial parts of *Orobanche crenata* in 1993.[5] In fact, cre-

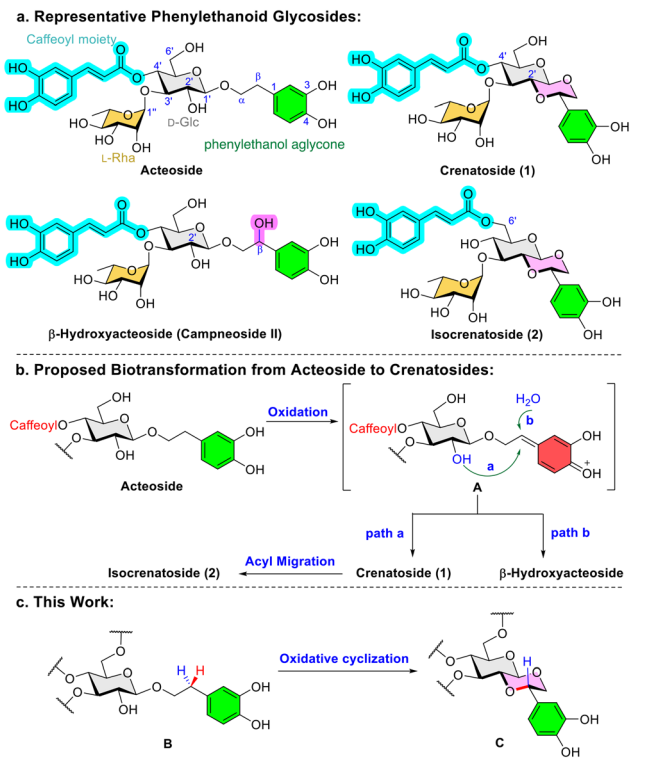

Fig. 1 (a) Representative phenylethanoid glycosides. (b) Proposed biotransformation from acteoside to crenatosides. (c) Oxidative cyclization approach for constructing the 1,4-dioxane skeleton in this work.

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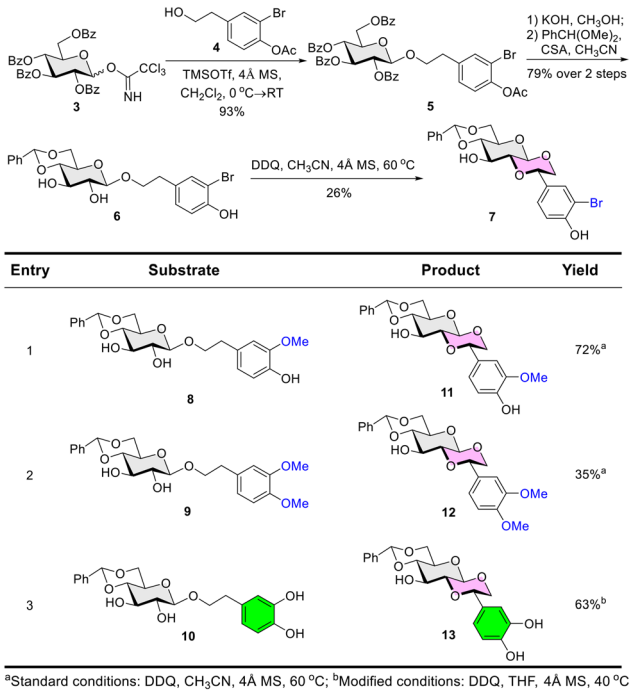
natoside (**1**) is the first phenylethanoid glycoside disclosed to possess a fused dioxane skeleton, characterized by an ether linkage connecting O′-2 of glucose to the benzylic carbon of 3,4-dihydroxyphenylethanol aglycone. It was suggested that 1,4-dioxanyl and caffeoyl moieties play pivotal roles in the selective inhibition of neuraminidase (NA) of influenza A virus (H1N1).[6] In 1998, isocrenatoside (**2**) was identified as the 6′-*O*-caffeoyl isomer of crenatoside from the fresh whole plant of *Orobanche coerulescens*.[7]

All these four PhGs share common structural features, including the central D-glucose, 3-*O*-L-rhamnose, the 4-*O*-caffeoyl moiety, and 3,4-dihydroxyphenylethyl aglycone. The primary difference lies in the oxidation state of the benzylic carbon in 3,4-dihydroxyphenylethanol aglycone. Among them, acteoside is the most abundant disaccharide caffeoyl ester PhG.[8] We hypothesize that the minor β-hydroxyacteoside and crenatoside (**1**) could be derived from acteoside through an oxidative transformation (Fig. 1b). Thus, acteoside underwent phenol oxidation to generate 2,5-cyclohexadienone intermediate **A**; subsequent addition of water led to the formation of β-hydroxyacteoside. Alternatively, an intramolecular nucleophilic addition of glucose 2′-OH resulted in the formation of 1,4-dioxane fused crenatoside (**1**). Moreover, the caffeic acid moiety could undergo acyl migration from glucose O-4′ to O-6′ to afford isocrenatoside (**2**). Over the past few decades, only acteoside and a few analogous PhGs have been synthesized.[9,10] It is noteworthy that Zeng *et al.* constructed a glucose fused 1,4-dioxane skeleton *via* a NaH-mediated intramolecular alkylation of glucose O′-2 with the bromide of the phenylethanol moiety, albeit without stereoselectivity.[6] Herein, we present the first synthesis of crenatoside (**1**), isocrenatoside (**2**) and their analogs through an oxidative cyclization approach (Fig. 1c).[11]

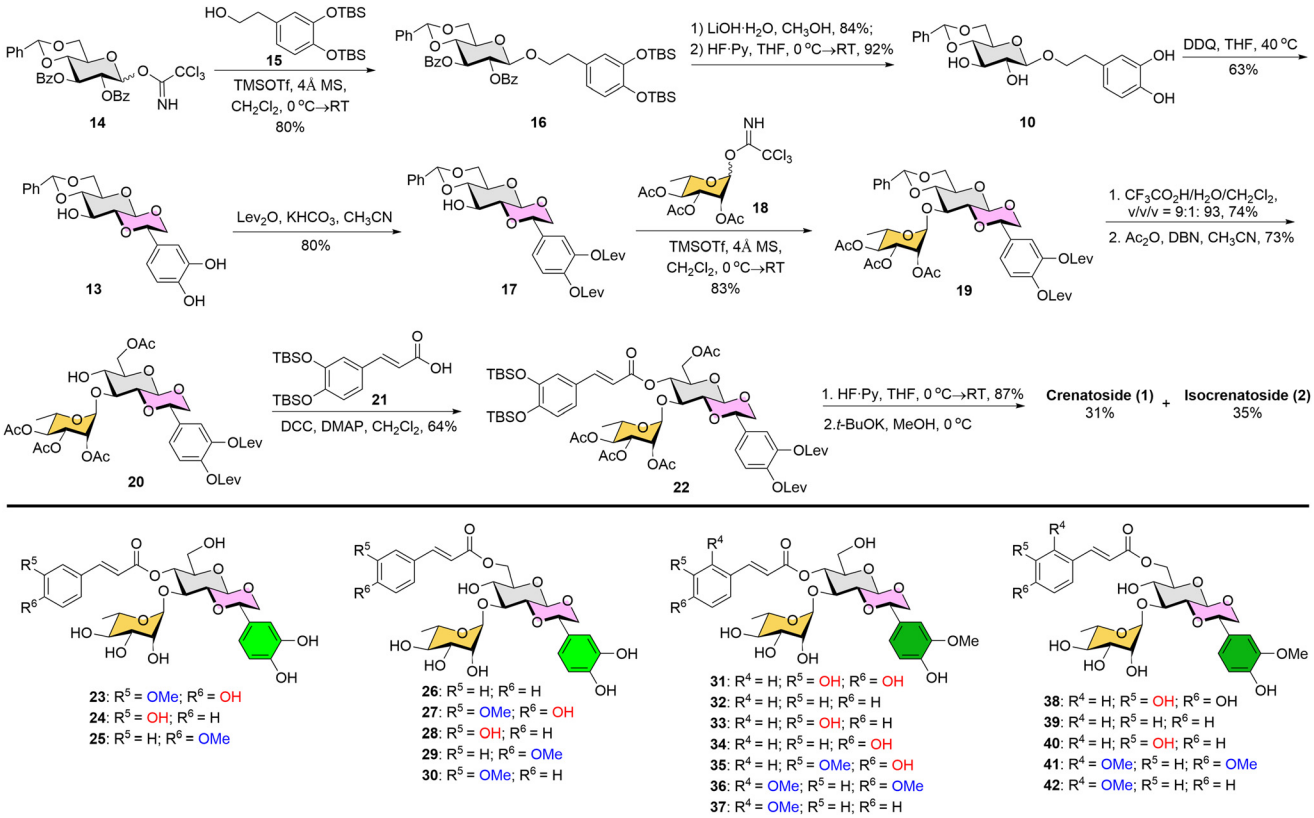


Scheme 1 Synthesis of 1,4-dioxane fused glycosides **7**, **11–13**.

Results and discussion

We embarked on an examination of the bio-inspired oxidative cyclization step to construct the glucose fused 1,4-dioxane skeleton (Scheme 1). Thus, perbenzoyl glucopyranosyl trichloroacetimidate **3** was coupled with 3-bromo-4-acetoxy-phenylethanol **4** under the catalysis of TMSOTf to provide phenylethanoid glycoside **5** (93%), which was then transformed to the desired cyclization precursor **6** by the removal of benzoyl and acetyl groups followed by the formation of 4,6-*O*-benzylidene. After a thorough examination of various reaction parameters, including benzoquinone, solvent, and concentration, we successfully obtained the desired 1,4-dioxane glycoside **7**, employing 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) as an oxidant and acetonitrile as a solvent at 60 °C, albeit with a moderate 26% yield (see the ESI† for details).[11] We attributed the low yield to the electron-withdrawing properties of the bromide substituent in the phenyl moiety, which was installed to facilitate later-stage derivatization. Thus, we turned our attention to phenylethanoid glycosides (*i.e.*, **8–10**), which bear electron rich phenyl moieties. Indeed, under similar conditions, the cyclization reaction of 3-methoxy-4-hydroxy-phenylethanoid glycoside **8** proceeded smoothly, leading to **11** in a good 72% yield. When the 4-hydroxy group was replaced with the 4-methoxy group (in **9**), the yield of the oxidative cyclization was reduced to 35%. Considering that the realistic structures of crenatoside (**1**) and isocrenatoside (**2**) feature 3,4-dihydroxy substituents, we prepared 3,4-dihydroxyphenylethanoid glycoside **10**. Nevertheless, compound **10** was found poorly soluble in acetonitrile due to the presence of four hydroxyl groups. Fortunately, the oxidative cyclization of **10** could be successfully carried out in tetrahydrofuran, affording the desired 1,4-dioxane fused glycoside **13** in a satisfactory 63% yield.

To achieve the total synthesis of crenatosides, 2,3-dibenzoyl-4,6-*O*-benzylidene glucosyl trichloroacetimidate **14** was coupled with 3,4-OTBS-phenylethanol **15** under the conventional conditions to deliver phenylethanoid glycoside **16** on a decagram scale (Scheme 2). Subsequent saponification and desilylation generated ring-closing precursor **10** (7.4 g). Utilizing the previously optimized DDQ-mediated oxidative cyclization in THF, the desired 1,4-dioxane fused glycoside **13** was obtained in 63% yield (on a 1.4 g scale). To selectively protect the phenolic hydroxyls, levulinic anhydride and potassium bicarbonate were used to give **17** (80%), which bears free 3′-OH.[12] Under the conventional conditions (TMSOTf, 4 Å MS, CH_2Cl_2, 0 °C → RT), monosaccharide **17** was coupled with peracetylrhamnosyl trichloroacetimidate **18** to provide α-linked disaccharide **19** in 83% yield. Following the acidic removal of the 4,6-*O*-benzylidene group in **19**, we selectively masked the 6-OH with acetic anhydride in the presence of diazabicyclononene (DBN), giving 4′-ol **20**.[13] Subsequent esterification of the remaining 4-OH with TBS-protected caffeic acid led to the fully



Scheme 2 Synthesis of crenatoside (**1**), isocrenatoside (**2**), and their analogs (**23–42**).

protected crenatoside **22** in 64% yield. The final stage for the synthesis of crenatoside **1** called for selective deprotection of TBS and acyl groups. We achieved delicate removal of TBS groups in **22** using Olah's reagent in THF. Finally, selective deacylation with potassium *t*-butoxide at 0 °C furnished crenatoside **1** (31%), along with isocrenatoside **2** (35%).[14] Evidently, the resultant crenatoside **1** underwent caffeinic migration from O-4 to O-6 under basic conditions to lead to isocrenatoside **2**. The NMR spectroscopic data of the synthetic crenatosides were virtually identical to those recorded for the authentic phenylethanoid glycosides (see Tables S3–S6† for details).

At this juncture, we shifted our focus towards preparing analogs of crenatosides and conducting bioactivity tests (see the ESI† for details). Leveraging common intermediate **20**, we conveniently obtained crenatoside analogs **23–25** and isocrenatoside analogs **26–30**. Employing 1,4-dioxane fused glycoside **11** as the precursor, we successfully executed a collective synthesis of analogs **31–42** in a similar approach. These 22 phenylethanoid glycosides (**1**, **2**, and **23–42**) were subjected to bioactivity assays (see Tables S7–S9† for details). Upon bioactivity screening, most of the 22 phenylethanoid glycosides were tested for anti-inflammatory activity in the lipopolysaccharide stimulated mouse macrophage, RAW 264.7 cells, which could reduce the production of tumor necrosis factor (TNF)-α in the cells at 20 μM. Additionally, these phenylethanoid glycosides exhibited immunosuppressive activity, in which compound **36** exhibited the most potent immunosuppressive activity, with an IC_{50} value of 19.9 μM on B lymphocyte proliferation responses. Some of the crenatoside derivatives (*i.e.*, **27** and **36**) possessed better biological activities compared to crenatoside (**1**) and isocrenatoside (**2**). These findings suggest that mild structural alterations, such as the substituents on the benzene rings, could significantly impact their bioactivities.

Conclusions

In summary, we have achieved the first chemical synthesis of phenylethanoid glycosides, crenatoside (**1**) and isocrenatoside (**2**), which bear a characteristic glucose fused 1,4-dioxane motif. By utilizing DDQ as an oxidant, the bio-inspired oxidative cyclization reactions could proceed effectively, resulting in the formation of 1,4-dioxane structures. The successful application of a range of substituted cinnamyl and hydroxyphenylethyl building blocks has enabled the synthesis of 20 crenatoside analogs, which demonstrated that mild structural alterations on the peripheral benzene rings could significantly impact their bioactivities. The availability of these 1,4-dioxane fused glycosides paves the way for further biological and pharmacological studies on PhGs.

Data availability

The data underlying this study are available in the published article and its ESI.†

ESI statement:

The ESI is available free of charge on the RSC Publications website.

Experimental details and NMR spectra are available in PDF.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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References

1 C. Jiménez and R. Riguera, Phenylethanoid glycosides in plants: structure and biological activity, *Nat. Prod. Rep.*, 1994, **11**, 591–606.

2 (*a*) G. Fu, H. Pang and Y. H. Wong, Naturally occurring phenylethanoid glycosides: potential leads for new therapeutics, *Curr. Med. Chem.*, 2008, **15**, 2592–2613; (*b*) X.-Y. Tian, M.-X. Li, T. Lin, Y. Qiu, Y.-T. Zhu, X.-L. Li, W.-D. Tao, P. Wang, X.-X. Ren and L.-P. Chen, A review on the structure and pharmacological activity of phenylethanoid glycosides, *Eur. J. Med. Chem.*, 2021, **209**, 112563.

3 (*a*) M. L. Scarpati and F. Monache, Isolation from *Verbascum sinuatum* of two new glucosides, verbascoside and isoverbascoside, *Ann. Chim.*, 1963, **53**, 356–367; (*b*) L. Birkofer, C. Kaiser and U. Thomas, Acteosid und neoacteosid; zuckerester aus *Syringa vulgaris* (L.), *Z. Naturforsch., B: J. Chem. Sci.*, 1968, **23**, 1051–1058; (*c*) Y. Xiao, Q. Ren and L. Wu, The pharmacokinetic property and pharmacological activity of acteoside: a review, *Biomed. Pharmacother.*, 2022, **153**, 113296.

4 S. Kitagawa, H. Tsukamoto, S. Hisada and S. Nishibe, Studies on the Chinese crude drug "Forsythiae fructus." VII. A new caffeoyl glycoside from *Forsythia viridissima*, *Chem. Pharm. Bull.*, 1984, **32**, 1209–1213.

5 M. S. Afifi, M. F. Lahloub, S. A. El-Khayaat, C. G. Anklin, H. Rüegger and O. Sticher, Crenatoside: a novel phenylpropanoid glycoside from *Orobanche crenata*, *Planta Med.*, 1993, **59**, 359–362.

6 B.-L. Chen, Y.-J. Wang, H. Guo and G.-Y. Zeng, Design, synthesis, and biological evaluation of crenatoside analogues as novel influenza neuraminidase inhibitors, *Eur. J. Med. Chem.*, 2016, **109**, 199–205.

7 T. Murayama, Y. Yanagisawa, A. Kasahara, K. I. Dnodera, M. Kurimoto and M. Ikeda, A novel phenylethanoid, isocrenatoside isolated from the whole plant of *Orobanche coerulescens*, *Nat. Med.*, 1998, **52**, 455–458.

8 Y. Zhou, J. Zhu, L. Shao and M. Guo, Current advances in acteoside biosynthesis pathway elucidation and biosynthesis, *Fitoterapia*, 2020, **142**, 104495.

9 (*a*) T. Kawada, R. Asano, S. Hayashida and T. Sakuno, Total synthesis of the phenylpropanoid glycoside, acteoside, *J. Org. Chem.*, 1999, **64**, 9268–9271; (*b*) H. I. Duynstee, M. C. de Koning, H. Ovaa, G. A. van der Marel and J. H. van Boom, Synthesis of verbascoside: a dihydroxyphenylethyl glycoside with diverse bioactivity, *Eur. J. Org. Chem.*, 1999, 2623–2632; (*c*) G. Gu, Y. Zhao and Z. Guo, Synthesis of Leonosides E and F derived from *Leonurus japonicas* Houtt, *Carbohydr. Res.*, 2013, **380**, 174–180; (*d*) S. K. Mulani, J.-H. Guh and K.-K. T. Mong, A general synthetic strategy and the anti-proliferation properties on prostate cancer cell lines for natural phenylethanoid glycosides, *Org. Biomol. Chem.*, 2014, **12**, 2926–2937; (*e*) P. Shu, X. Xiao, Y. Zhao, Y. Xu, W. Yao, J. Tao, H. Wang, G. Yao, Z. Lu, J. Zeng and Q. Wan, Interrupted Pummerer reaction in latent-active glycosylation: glycosyl donors with a recyclable and regenerative leaving group, *Angew. Chem., Int. Ed.*, 2015, **54**, 14432–14436; (*f*) D. T. Khong and Z. M. A. Judeh, Short synthesis of phenylpropanoid glycosides calceolarioside A and syringalide B, *Synlett*, 2018, 1079–1083; (*g*) X. Zhang, Y. Yang, F. Wang, Z. Zhou, H. Zhang and Y. Zhu, An approach to the synthesis of electron-rich and hindered esters and its application to the synthesis of acteoside, *Org. Lett.*, 2021, **23**, 9210–9215; (*h*) H. Dong, W. Du, Z. Yao, M. Wu, H. Luo, Y. He and S. Cao, First total syntheses of two natural glycosides, *Carbohydr. Res.*, 2021, **499**, 108200; (*i*) M. Mastihubová and P. Kis, A sustainable approach to phenylethanoid glycopyranosides: Study of glycosylations promoted by zinc salts, *Sustainable Chem. Pharm.*, 2021, **24**, 100537.

10 (*a*) Z. Hu, P. Xu and B. Yu, A new approach to the synthesis of acteoside, *Chin. J. Org. Chem.*, 2020, **40**, 3439–3445; (*b*) Z. Hu, P. Xu, B. Wei and B. Yu, Total synthesis of phenylpropanoid glycosides, acteoside, isoacteoside and ligupurpuroside J, *Chem. J. Chin. Univ.*, 2020, **41**, 1708–1720; (*c*) Y. Cheng, Y. Xia, Z. Yuan, H. Li, J. Wang, Y. Wang, C.-G. Yang and B. Yu, Expeditious synthesis of gwanakoside A and the chloronaphthol glycoside congeners, *Org. Lett.*, 2024, **26**, 2425–2429; (*d*) D. Zhu and B. Yu, Synthesis of the diverse glycosides in traditional Chinese medicine, *Chin. J. Chem.*, 2018, **36**, 681–691.

11 (*a*) Y. Oikawa, T. Nishi and O. Yonemitsu, Kinetic acetalization for 1,2- and 1,3-diol protection by the reaction of *p*-methoxyphenylmethyl methyl ether with DDQ, *Tetrahedron Lett.*, 1983, **24**, 4037–4040; (*b*) F. Yang, G. Lian

and B. Yu, Synthesis of raphanuside, an unusual oxathiane-fused thioglucoside isolated from the seeds of *Raphanus sativus L.*, *Carbohydr. Res.*, 2010, **345**, 309–314.

12 G. Macchione, S. Maza, M. Mar Kayser, J. L. de Paz and P. M. Nieto, Synthesis of chondroitin sulfate oligosaccharides using N-(tetrachlorophthaloyl)- and N-(trifluoroacetyl) galactosamine building blocks, *Eur. J. Org. Chem.*, 2014, 3868–3884.

13 B. Ren, M. Zhang, S. Xu, L. Gan, L. Zhang and L. Tang, DBN-catalyzed regioselective acylation of carbohydrates and diols in ethyl acetate, *Eur. J. Org. Chem.*, 2019, 4757–4762.

14 T. Kawada, R. Asano, K. Makino and T. Sakuno, Synthesis of isoacteoside, a dihydroxyphenylethyl glycoside, *J. Wood Sci.*, 2002, **48**, 512–515.