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Synthesis and cardiac activity evaluation of the proposed structures of fuzinoside

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ABSTRACT

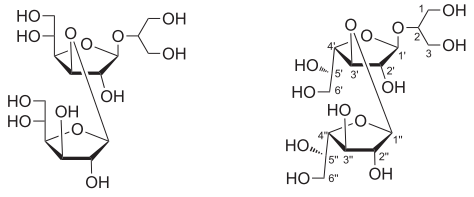
In order to revise the structure of fuzinoside and investigate its cardiac activity, four proposed structures (**2**–**5**) with different forms and linkage positions of the disaccharide were synthesized through 10 ~ 14 chemical steps from the commercially available D-galactose. None of the synthetic glycosides were consistent with the natural one by comparison of their NMR data, indicating that the true structure of fuzinoside remains to be confirmed. Nevertheless, cardiac activity evaluation in the isolated bullfrog heart assay has shown that glycerol β-D-galctofuranosyl-(1→2)-β-D-galactofuranoside (**2**) is a potential cardiotonic agent.

1. Introduction

The lateral roots of *Aconitum carmichaelii* Debeaux (known as 'Fuzi' in China) have been used as a traditional Chinese medicine, with a wide range of pharmacological activities including anti-inflammation, analgesic, anti-tumor, and cardiac effects, etc.[1] As the predominant constituents, diterpenoid alkaloids are responsible for most of the activities of *A. carmichaelii*.[2] In our continuous investigations on the bioactive components of the genera *Aconitum* and *Delphinium*, we have been particularly interested in the cardioactive compounds from the species of *A. carmichaelii* recently.[3,4]

Except for the well-known diterpenoid alkaloids, a cardiotonic glycoside, fuzinoside (**1a**), was isolated by Xu and co-workers from the non-alkaloidal fractions of *A. carmichaelii* in 2004, the structure of which was elucidated as glycerol β-D-galactofuranosyl-(1→3)-β-D-galactofuranoside.[5] It should be noted that the structure of fuzinoside should be redrawn as **1b** in accordance with its name (Fig. 1). The apparent cardiotonic effect of fuzinoside was observed on isolated working hearts of guinea pigs, by significantly increasing the data of left ventricular systolic pressure (LVSP) and maximal rise or decline rate of left ventricular pressure (±dp/dtmax). From the viewpoint of mechanism, its positive inotropic action might be generated through the activation of calcium ion channel based on the in vivo studies.[5b,c] Due to its scarcity from natural sources and intriguing biological profile, we embarked on a total synthesis of fuzinoside for an in-depth exploration of the cardiotonic effects and structure-activity relationships.

Our previous endeavors have resulted in the synthesis of **1b** through two different strategies.[6] Nevertheless, the NMR data for the synthetic fuzinoside **1b** are not in consistent with those reported in the literature,[5] indicating the originally assigned structure of this molecule might be incorrect and needs to be revised. On the basis of the hydrolysis experiment carried out from the isolation paper,[5] it was clear that fuzinoside consisted of two β-galactoses and one glycerol. Consequently, several possible structures for this glycoside with alternative forms and linkage positions of the disaccharide were proposed (Fig. 2): glycerol β-D-



a Fuzinoside (the reported structure in the literature)[5]

b Fuzinoside (the redrawn structure according to its name)[5]

Fig. 1. The reported and redrawn structures for fuzinoside according to the literature.

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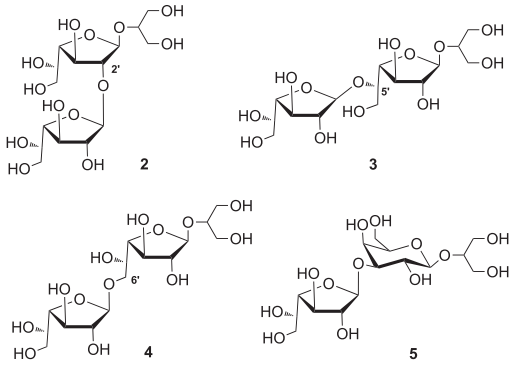


Fig. 2. The proposed structures for fuzinoside.

galctofuranosyl-(1→2)-β-D-galactofuranoside (**2**), glycerol β-D-galctofuranosyl-(1→5)-β-D-galactofuranoside (**3**), glycerol β-D-galctofuranosyl-(1→6)-β-D-galactofuranoside (**4**), and glycerol β-D-galctofuranosyl-(1→3)-β-D-galactopyranoside (**5**). In this paper, we wish to report the chemical synthesis of the proposed structures of fuzinoside. Cardiac activity of all the prepared final products and analogues was evaluated in the isolated bullfrog assay, among which compound **2** displayed strong cardiotonic effects compared to the positive drug lanatoside C.[7]

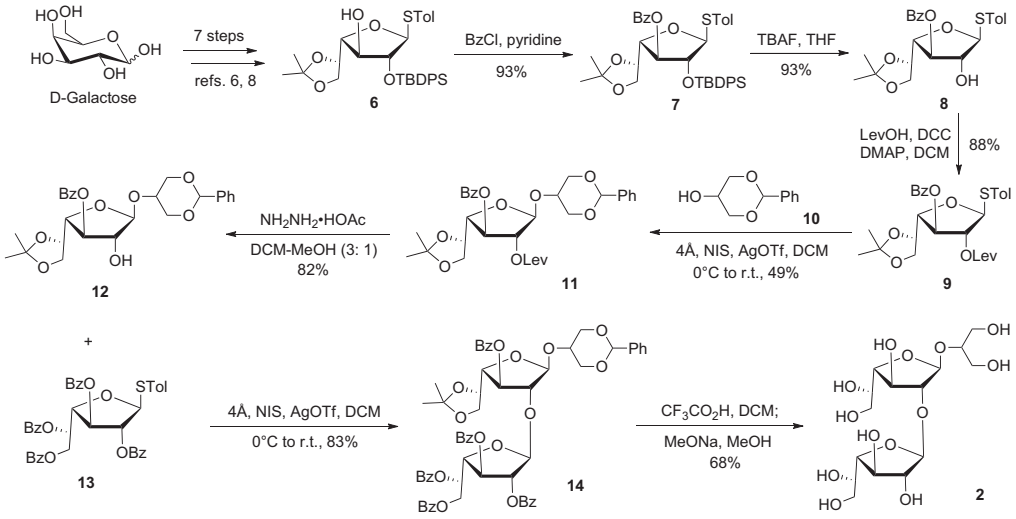
2. Results and discussion

In an attempt to understand the structure of fuzinoside, we synthesized four proposed glycosides (**2**–**5**) with different forms and linkage positions of the disaccharide. In this context, the so-called AB+C approach was applied based on our previous studies,[6] that is, preparation of the glycerol-β-D-galactose via *O*-glycosylation first, followed by incorporation of another molecular of D-galactose.

The synthesis of compound **2** commenced with the preparation of donor **9** (Scheme 1). Specifically, the known thioglycoside **6** (-STol: *p*-tolylthio) was accessed in 40% overall yield from D-galactose employing a six-step procedure, according to the method described in the literature.[6,8] Protection of C3′-OH in **6** by reacting with benzoyl chloride catalyzed by pyridine gave compound **7** in 93% yield. Treating the latter with TBAF followed by reacting with levulinic acid (LevOH, 4-oxopentanoic acid) in the presence of DMAP and DCC afforded the fully protected derivative **9** in 82% yield over two steps. Having the donor **9** in hand, *O*-glycosylation with alcohol **10**[9] occurred in moderate yield under the conditions of *N*-iodosuccinimide (NIS) and silver triflate (AgOTf), leading to glycerol galactoside **11**. Its NMR spectra exhibited characteristic signals for anomeric H-1′ (δ_H 5.55 s) and C-1′ (δ_C 105.2 d), suggesting the *trans*-relationship of H-1′ and H-2′, and the existence of α-configuration of H-1′.[10,11] Removal of the levulinyl (Lev) protecting group in compound **11** with hydrazine acetate gave rise to compound **12** smoothly. Reaction of accepter **12** with donor **13**[8] in the presence of NIS/AgOTf afforded disaccharide **14** with a 1→2 glycosidic bond in 83% yield. The diagnostic peaks for anomeric H-1″(δ_H 5.69 s) and C-1″(δ_C 106.2 d) (δ_C 109.7 after full deprotection) from the NMR spectra of advanced intermediate **14** demonstrated the *trans*-relationship of H-1″ and H-2″, as well as an α-configuration of H-1″.[10,11] Ultimately, global deprotection of **14** through acid and base, followed by purification by Sephadex LH-20 column yielded **2** in a total yield of 68% for two steps.

The preparation of disaccharides **3** and **4** relied on two known thioglycosides **15** and **16**.[8] As depicted in Scheme 2, subjecting thioglycoside **15** to LevOH in the presence of DMAP and DCC afforded the fully protected derivative **17** in 94% yield. Condensation of accepter **10** with donor **17** mediated by NIS/AgOTf generated compound **19** in 72% yield. Its ^{1}H NMR spectrum showed a singlet signal at δ_H 5.54 for H-1′, indicating the *trans*-relationship of H-1′ and H-2′, and an α-orientation of H-1′.[10,11] Deprotection of the Lev group in compound **19** with hydrazine acetate yielded compound **21** (93%), revealing the free hydroxyl group at C-5′. At this juncture, reaction of **21** with thioglycoside **13** in the presence of NIS and AgOTf afforded disaccharide **23** with a 1→5 glycosidic bond in 75% yield. The β-orientation of the newly formed glycosidic bond was confirmed by the NMR spectra of **23**, which featured typically anomeric H-1″ (δ_H 5.78 s) and C-1″ (δ_C 105.0 d) signals. The *trans*-relationship of H-1″ and H-2″ could also be concluded from the observations. Deprotection of **23** through similar acidic and basic conditions, respectively, provided disaccharide glycoside **3**.

In a similar manner, the synthesis of disaccharide **4** started with the coupling between **18** and **10** to give **20** in 70% yield (Scheme 2). Its ^{1}H NMR spectrum showed a singlet signal at δ_H 5.62 for H-1′, suggesting the *trans*-relationship of H-1′ and H-2′, and α-orientation of H-1′. Removal of the Lev protecting group in compound **20**



Scheme 1. Synthesis of glycerol β-D-galctofuranosyl-(1→2)-β-D-galactofuranoside (**2**).