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ORIGINAL PAPER

Total syntheses of schizandriside, saracoside and (±)-isolariciresinol with antioxidant activities

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Abstract
Lignans are widely distributed in plants and exhibit significant pharmacological effects, including anti-tumor and antioxidative activities. Here, we describe the total synthesis of schizandriside (**1**), a compound we previously isolated from *Saraca asoca* by monitoring antioxidative activity using the 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay. Starting from a tandem Michael-aldol reaction, the lignan skeleton was synthesized in 6 steps, including a cyclization step. To determine the stereochemistry of **1**, we synthesized the natural product (±)-isolariciresinol (**18**) from alcohol **17**. Comparison of the spectral data showed good agreement. Glycosylation was investigated using four different glycosyl donors. Only the Koenigs–Knorr condition using silver trifluoromethanesulfonate with 1,1,3,3-tetramethylurea provided the glycosylated product. Deprotection and purification using reverse-phase high-performance liquid chromatography gave schizandriside (**1**) and its diastereomer saracoside (**2**). Synthesized **1**, **2** and **18** showed antioxidant activity with IC_{50} = 34.4, 28.8, 53.0 μM, respectively.

Keywords Lignan glycoside · Antioxidant activity · Total synthesis · Natural product

Introduction

Lignans are widely distributed in plants, and new related compounds continue to be isolated [1]. Although the biological functions of lignans remain unclear, some significant pharmacological effects have been revealed, including antitumor and antioxidative activities. We previously isolated schizandriside (**1**) from *Saraca asoca* by monitoring antioxidative activity determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay [2].

The attractive biological activities of aryltetralin lignan lactones have long resulted in significant efforts towards their synthesis. Two major approaches can be used to synthesize the aryltetralin lactone skeleton [3]—the Diels–Alder reaction and the Michael-aldol reaction. However, the synthesis of schizandriside (**1**) or lignan glycosides substituted on C9 has not been reported to date despite schizandriside (**1**) being first isolated in 1979 [4]. Although lignan glycosides can be isolated in adequate amounts for biological assays, a synthetic approach is required for evaluating bioactivity systematically. Here, we report the total synthesis of schizandriside (**1**) and saracoside (**2**) and the evaluation of their antioxidant activities.

Results and discussion

Our retrosynthetic analysis is based on the Michael-aldol reaction, which is the most common approach for such syntheses (Scheme 1). We planned to perform the glycosylation reaction last to facilitate the synthesis of derivatives on C9. Glycoside precursor **4** was synthesized from alcohol **5** via a cyclization step.

Starting from known compounds lactone **9**, dithiane **10** [5], and aldehyde **11** [6], the Michael-aldol reaction gave alcohol **12** in 65% yield (dr = 1.8:1, determined by NMR, Scheme 2). We performed the next reaction using a diastereomeric mixture. Reaction using $NaBH_4$ and $NiBr_2$ [7, 8] reductively cleaved the dithiane substituent successfully

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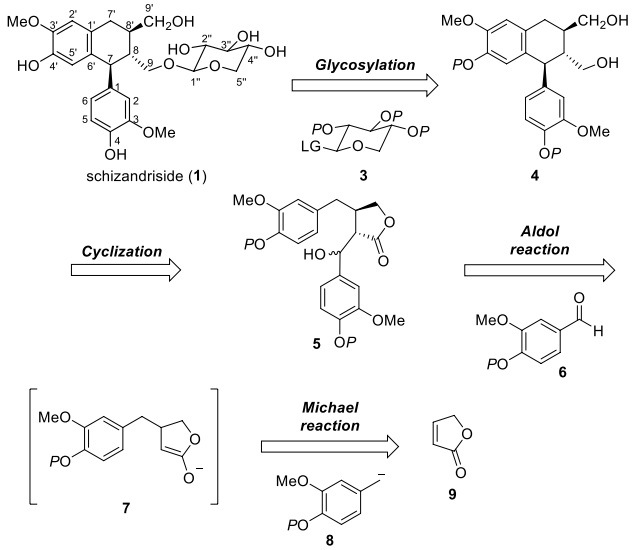
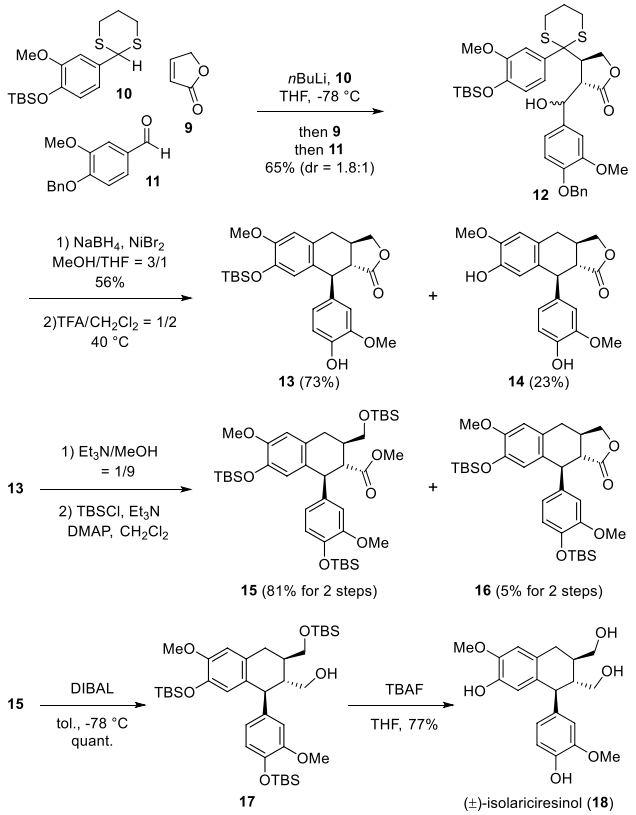


Scheme 1 Retrosynthetic analysis of schizandriside (**1**)

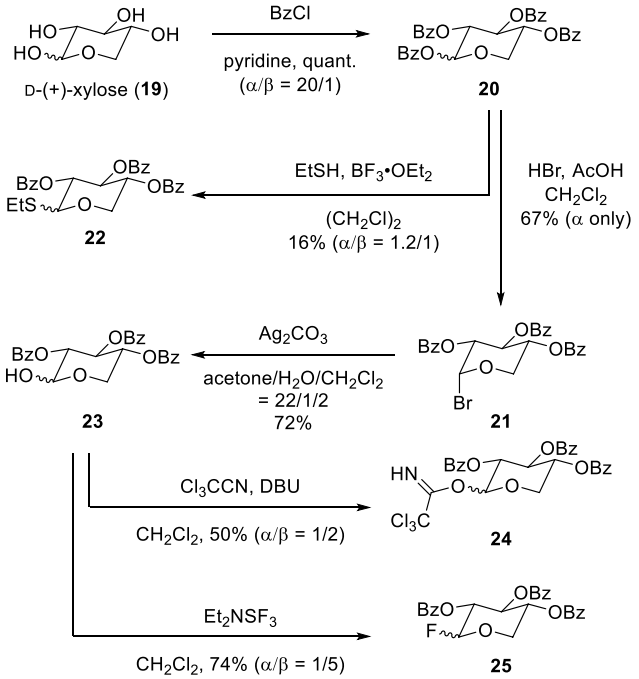


Scheme 2 Synthesis of alcohol **17** and (±)-isolariciresinol **18**. *DIBAL* diisobutylaluminium hydride, *TBAF* tetra-*n*-butylammonium fluoride

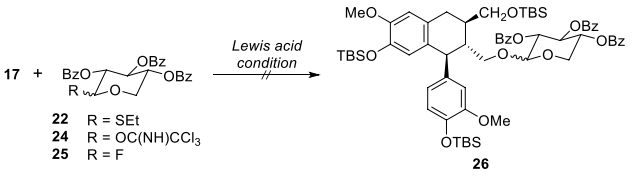
and removed a benzyl group. Cyclization under acidic conditions gave **13** as a single diastereomer, with diol **14** as a byproduct. Next, we attempted acid-catalyzed methanolysis of the lactone unit while preventing isomerization at C8 but were unable to establish successful reaction conditions. Methanolysis with triethylamine (Et_3N) followed by *tert*-butyldimethylsilyl (TBS) protection gave **15**. Partial lactonization occurred during the protection step to give **16**. However, lactone **16**, as well as diol lactone **14**, can be converted to **15** by following the same synthesis steps. Reduction by $LiAlH_4$ caused removal of the TBS group and thus diisobutylaluminium hydride was used to obtain alcohol **17**. To determine the stereochemistry, we removed the TBS groups from alcohol **17** to give aglycon **18**, which is the natural product (±)-isolariciresinol. The ^1H and ^{13}C NMR spectra of aglycon **18** showed good agreement with reported values [9], indicating that the stereochemistry of the synthesized compound is correct.

For the glycosyl donor, we initially planned to use acetate to protect the hydroxy groups of D-(+)-xylose **19** but the acetyl group migrated to alcohol **17**. Therefore, the protective group was changed to the benzoyl group (Bz, Scheme 3). After protection with benzoyl chloride, **20** was converted to bromide **21** and thioglycoside **22**. Alcohol **23** was synthesized from bromide **21**. Reactions to give trichloroacetimidate **24** and fluoride **25** resulted in moderate yields.

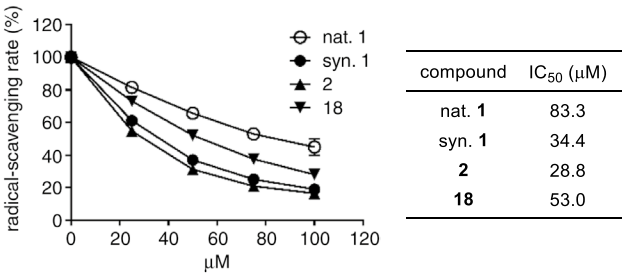
We investigated glycosylation conditions using four different glycosyl donors. Trials without molecular sieves 4A gave complex mixtures. Use of the boron trifluoride diethyl ether complex or trimethylsilyl trifluoromethanesulfonate as an activator for glycosyl donors **22**, **24**, and **25** caused removal of the TBS groups (Scheme 4). Only the



Scheme 3 Synthesis of glycosyl donors. *DBU* 1,8-diazabicyclo[5.4.0]undec-7-ene



Scheme 4 Attempted glycosylation with glycosyl donor **22**, **24** and **25**



compound	IC_{50} (μM)
nat. **1**	83.3
syn. **1**	34.4
2	28.8
18	53.0

Fig. 1 Evaluation of antioxidant activity using the DPPH radical scavenging assay

Koenigs–Knorr condition using silver trifluoromethanesulfonate with 1,1,3,3-tetramethylurea resulted in glycosylation (Scheme 5). We could not separate the four possible diastereomers at this point. The coupling constant indicated that two of the major diastereomers have β glycoside linkages.

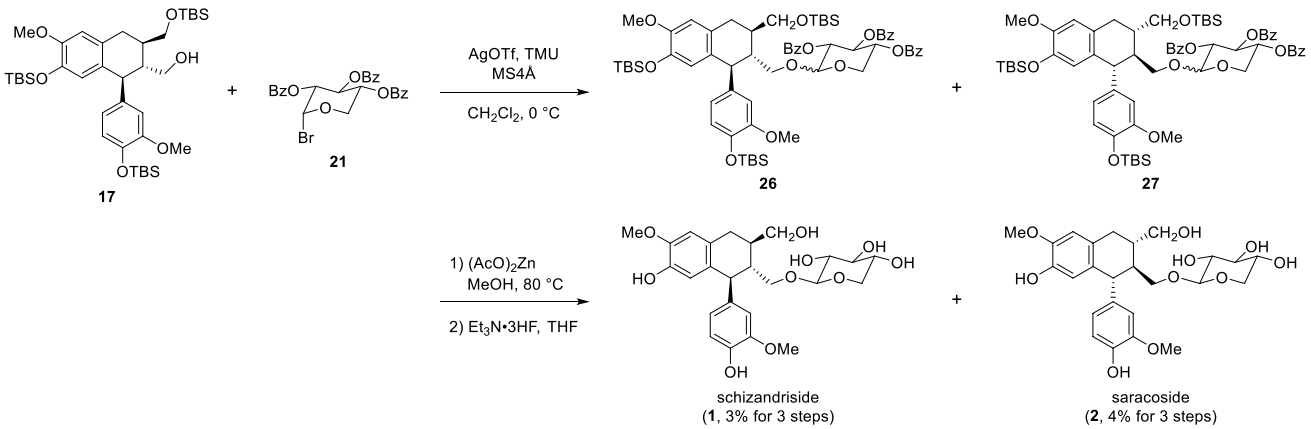
Unfortunately, the hydrolysis of benzoates **26** and **27** under basic conditions was unsuccessful. Notably, deprotection of the C2″ hydroxy group was difficult, probably due to steric hindrance. Only the use of zinc acetate [10] as a catalyst gave the desired result (Scheme 5). Since we could not isolate the diastereomers at this step, we moved on to the last step. After removal of the TBS groups with $Et_3N{\cdot}3HF$, purification using high-performance liquid chromatography gave schizandriside (**1**) and the diastereomer saracoside (**2**) in 3 and 4% yield, respectively, after these three steps. The α glycoside yields were very low, and we could not isolate the compounds after deprotection. All spectral data of synthesized **1** and **2** showed good agreement with data of the corresponding natural compound schizandriside [2] and saracoside [11], respectively.

We performed DPPH radical scavenging assays using **1**, **2**, and aglycon **18** (Fig. 1). Synthesized **1** showed higher activity compared to the natural product, probably due to its purity (IC_{50} = 34.4 and 83.3 μM, respectively). Compound **2** showed activity (IC_{50} = 28.8 μM) that was similar to that of **1**. Aglycon **18** also showed activity (IC_{50} = 53.0 μM), indicating that the presence of xylose is not related to the antioxidant activity of lignans.

In conclusion, we synthesized schizandriside (**1**) and two other natural products, saracoside (**2**) and (±)-isolariciresinol (**18**). Synthesized **1**, **2** and **18** showed antioxidant activity with IC_{50} = 34.4, 28.8, 53.0 μM, respectively. We are currently synthesizing other derivatives.

Antioxidant assay

The antioxidant activity was evaluated according to a previous report [12]. Using a 96-well clear microplate, 10 μL of sample-DMSO solution was placed in each microwell. Quercetin (25 μM) was used as a positive control. DPPH-MeOH solution (190 μL, final concentration of DPPH: 200 μM) was added to each microwell and the microplate was then shaken for 30 min at room temperature using a microplate mixer. The absorbance was determined at 492 nm using a microplate reader (Thermo Scientific Multiskan FC). Each sample was measured in triplicate and the mean result was taken. The antioxidant activity was expressed in terms of IC_{50} (μM).



Scheme 5 Total synthesis of schizandriside (**1**). *TMU* 1,1,3,3-tetramethylurea, *THF* tetrahydrofuran

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