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Novel Synthesis of benzyl-Methoxyl Protected Aspalathin Analog *via* C-Glucosylation of Pentamethoxy Dihydropropane

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Abstract: The first report on a novel and efficient synthesis of benzyl-methoxy protected aspalathin derivative has been described via C-glucosylation of pentamethoxy dihydropropane. The synthesized compound was characterized by ^{1}H, ^{13}C NMR, COSY, and HSQC techniques.

Keywords: aspalathin;glucolysation;glycoside.

1. Introduction

Glycoside is a molecule that contains a sugar moiety attached to another moiety through a glycosidic bond *via* its anomeric (C-1') carbon. The sugar moiety is known as glycone and another moiety as the glycone part of the glycoside. Glycosides can be linked *via* oxygen- (an *O*-glycoside), nitrogen- (a glycosylamine), sulfur- (a thioglycoside), or carbon- (a *C*-glycoside) glycosidic bond. Glycosides show important biological functions. As polymers, they are an important store of energy in plants that serve as food sources for animals and humans. Oligomeric glycosides on mammalian cell surfaces play an important role in the immune system. The glycone moiety usually renders the glycoside more water-soluble. Thus, glycosylation makes metabolite toxins and other unwanted secondary metabolites in mammals water-soluble for excretion by the kidneys. Many secondary metabolites in plants are glycosides. Some of these are toxins that protect the plant against herbivores. Plethoras of other activities in mammalian and human biology has been demonstrated [1-8]. Flavonoids normally accumulate in plants as *O*-glycosylated derivatives. However, several species, including major cereal crops, predominantly synthesize flavone *C*-glycosides. These are stable to hydrolysis and are biologically active both in plants and mammals. Activities ascribed to these plant secondary metabolites include them functioning as antioxidants [9-10], insect feeding attractants [11], antimicrobial agents [12], promoters of mycorrhizal symbioses [13], and UV-protective pigments [14].

2. Materials and Methods

Aspalathin (1a) (Figure 1) (3'-*β*-D-glucopyranosyl-2', 3, 4, 4', 6'-pentahydroxydihydrochalcone) is a dihydrochalcone *C*-glucopyranoside. It was first characterized by Koeppen [15] and co-workers in 1965. Notably is the *β*-stereochemistry at the anomeric carbon. It occurs exclusively in leaves of *Aspalathus linearis* (rooibos) where it is the major component. It has recently received considerable interest due to its plasma sugar lowering properties [16-17]. Nothofagen (1b) (3'-*β*-D-glucopyranosyl-2', 4, 4', 6'-tetrahydroxydihydrochalcone) differs from aspalathin in the absence of the 3-hydroxy on the A-ring. It occurs in a much lower concentration in rooibos.

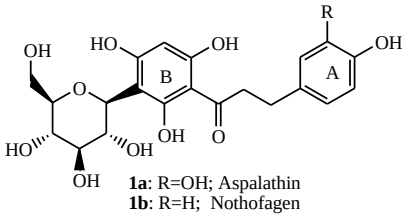
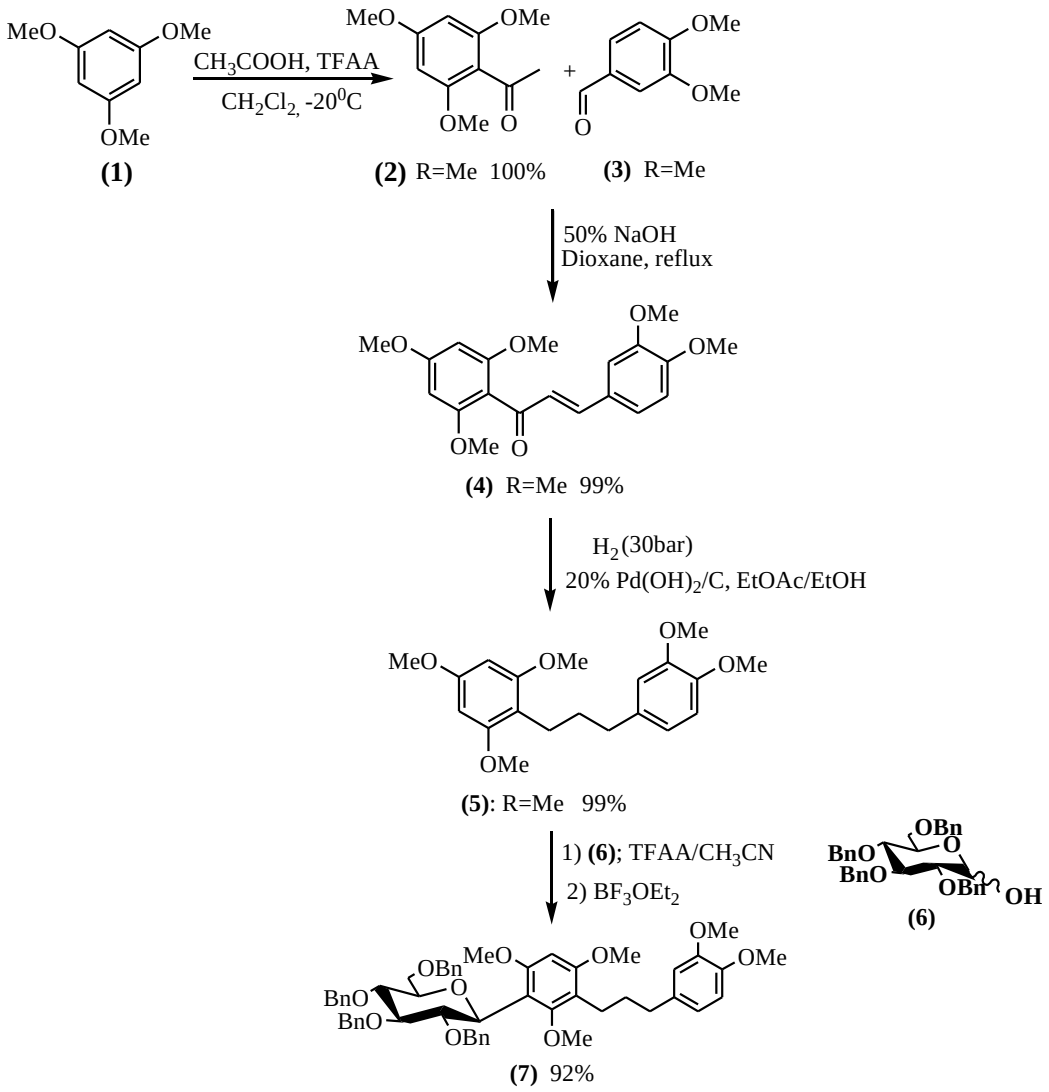


Figure 1. Structures of aspalathin (1a) and nothifagen (1b).



Scheme 1. Synthesis of diarylpropane (5) and *C*-diarylpropane-glycoside (7).

The isolation of naturally occurring *C*-aryl glycosides with important pharmacological properties [18-23] has prompted synthetic methods that are also relevant to the synthesis of

aspalathin and analogs. The *C*-glycosidic bond confers stability to O-glycosides to both enzymatic and chemical hydrolysis and, thus, probably contributes to enhanced bioavailability. However, it is much more difficult to form a carbon-carbon bond than an ether bond, and *C*-glycoside synthesis has remained a challenge. The regio-and stereoselective requirements of the *C*-aryl linkage are the additional complications.

In continuation of our previous work on the synthesis of some bioactive heterocyclic compounds [24-27], in the present work, we report synthesis and characterization of benzyl-methoxyl protected aspalathin analog via C-glucosylation of pentamethoxy dihydropropane (Scheme 1).

Table 1. High-pressure hydrogenation of fully OMe protected chalcone.

Substrate	Hydrogen Pressure (Bar/PSi)	10% Pd/C (Equivalent)	Solvents (EtOAc/MeOH (Ratio)	Time (h)	Yield (%)
4	30/435	(0.1)	(50:50)	24	(100)
4	R.T. Using H_2, Balloon	(0.2)	EtOAc/H_2O/Dil, HCl	Overnight	99%

Table 2. Synthesis of **(7)** *via C*-glycosylation of diarylpropane (5).

Entry	Sugar donor	Catalyst (equivalents)		Solvent	Temperature (°C)	Time (h)	Yield (%)
1	6	BF_3OEt_2 (2)		DCM	0	14	14
2	Sugar-1[#]	TMSOTf (2)		DCM	-10	8	23
3	Sugar-2[#]	BF_3OEt_2 (2)		DCM	-20	6	42
4	Sugar-3[#]	$SnCl_4$ (2)		DCM	0	7	30
5	6	TFAA* (3)	BF_3OEt_2 (2)	DCM	-12	6	59
6	6	TFAA* (3)	BF_3OEt_2 (2)	CH_3CN	-12	3	92

* Preactivation with TFAA was essential for the coupling reaction to take place.

[#]Sugar-1: 2, 3, 4, 6-tetra-O-benzylglucosyl acetate, Sugar-2: 2, 3, 4, 6-tetra-O-benzylglucosyl acetamide, Sugar-3: 2, 3, 4, 6-tetra-O-benzyl-glycopyranosyl fluoride.

C-aryl glycosylation has been reviewed by Palmacci [28] and Seeberger [29]. The substituents may influence glycosidic bond formation's regioselectivity on the aromatic ring and the reaction conditions such as the temperature and pressure employed. C-aryl glycosidic bond formation's stereoselectivity was influenced by the structure of carbohydrate moiety, e.g., neighboring group effects, anomeric effects, and synthetic conditions such as the choice of catalysts and solvents.

3. Results and Discussion

The methoxy-protected chalcone (4) was obtained in quantitative yields. High-pressure catalytic hydrogenative reduction of (4) gave the corresponding 1, 3-diarylpropane (5) quantitatively (Scheme 1) (Table 1). Salient in the NMR of (5) is the following: The absence of the two chalcone proton resonances in the ^1H NMR spectrum [H_α (d, 7.25 ppm, J = 15.5 Hz) and H_β (d, 6.88 ppm, J = 15.5 Hz) for corresponding chalcone, and the absence of the carbonyl resonance (δ = 196) in the ^{13}C NMR; The three propane CH_2 groups are represented in the ^1H NMR at 2.58 ppm (two overlapping benzylic CH_2 groups that integrate four hydrogen atoms) and 1.77 ppm (a multiplet that integrates two hydrogen atoms). They correspond to two cross-peaks at 35.5 and 31.1 ppm in the HSQC; The 1,3-diarylpropane-*C*-glycoside (7) was subsequently obtained in almost quantitative isolated yield (92%, Entry 6, Table 2) from (6) *via* the trifluoroacetic anhydride (TFAA) method described under approach 1 (preactivation of the anomeric OH with TFAA) (Scheme 1); The glycosyl fluoride and BF_3OEt_2 gave (7) in a yield of only 42% (Entry 3, Table 2). The *C*-diarylpropane-glycoside (7) was characterized by the following observations:

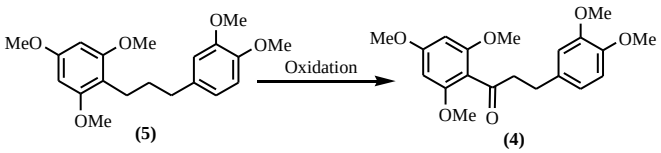
a) It has complex ^{1}H and ^{13}C NMR spectra at room temperature due to the expected rotational isomerism about the glycosidic carbon-carbon bond. Heating of the sample to 140°C in DMSO-d_6 was required for NMR elucidation.

b) The anomeric proton resonates at 4.73 ppm in the ^{1}H and its corresponding C at 74.2 ppm in the ^{13}C NMR spectra. These correspond with a carbon-carbon and not carbon-oxygen bond.

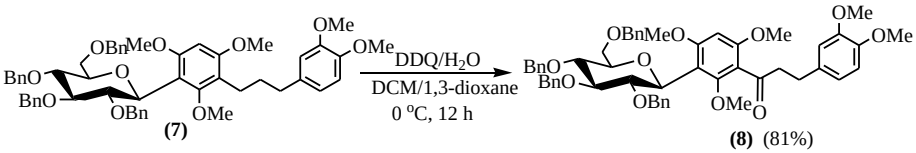
The J = 9.8 Hz coupling constant agrees with the required *β*-stereochemistry on the anomeric carbon.

An edited two-dimensional HSQC experiment; (CH and CH_3 cross-peaks having a different color from CH_2 cross-peaks) allows facile differentiation between the benzylic protons (CH_2) of the benzyl protection groups and the anomeric sugar proton (CH), all of which resonate in the range of δ 4.00-5.20 ppm.

We thus proved our hypothesis that the carbonyl group in chalcone or the phloroacetophenone moiety prevents *C*-glycosylation. Initial efforts to regenerate the carbonyl group *via* oxidation of (7) with Dess-Martin reagent, IBX (*o*-iodoxybenzoic acid), CAN (ceric ammonium nitrate), pyridine-dichromate, $Na_2Cr_2O_7$, and DDQ (dichlorodicyanoquinone) under anhydrous conditions failed. Upon further literature search and model reactions (Table 3) with the diarylpropane (5), we realized that the presence of water as a source of oxygen is essential for this oxidation. Upon treatment of (5) with DDQ in the presence of H_2O (1.5 equivalent), the corresponding dihydrochalcone (4) was obtained quantitatively at room temperature (Scheme 2). Compared to other oxidative conditions, it is mild with water as the oxygen source.



Scheme 2. Benzylic oxidation of the diarylpropane (5).



Scheme 3. Optimized synthesis of methyl-benzyl-protected aspalathin (8) *via* benzylic oxidation of (7).

Table 3. Oxidation condition for the synthesis of (5) at room temperature.

Entry	Oxidant	Solvents (2:1)	Time (h)	Yield (%)
1	IBX	Acetone*	14	0
2	Des-Martin	DCM*	8	0
3	CAN	DCM*	6	0
4	Pyridine-dichromate	DCM*	7	0
5	$Na_2Cr_2O_7$	DCM*	6	0
6	DDQ	DCM/Dioxane*	3	0
7	IBX	DCM#	14	14
8	Des-Martin	DCM#	8	23
9	CAN	DCM#	6	33
10	pyridine-dichromate	DCM#	7	30
11	$Na_2Cr_2O_7$	DCM#	6	59
12	DDQ	DCM/Dioxane#	3	92

* Anhydrous solvents were used in reactions

\# 1.5 equivalent of H_2O was present in the reaction solvents

Table 4. Optimized oxidation conditions for the synthesis of *C*-dihydrochalcone glycoside (7).

Entry	Oxidant (Eq)	Solvents (2:1)	Temp. (°C)	Time (h)	Yield %
1	DDQ (4)	DCM/Dioxane[#]	r.t.	2	44
2	DDQ (4)	DCM/Dioxane[#]	0	12	81

[#] 1.5 equivalent of H_2O was present in the reaction solvents

The same treatment of (7) with DDQ at room temperature under the conditions optimized for (5) yielded the expected dihydrochalcone-*C*-glycoside (8) in a 44% yield. Upon lowering the reaction temperature to 0 °C, the reaction proceeded slower but produced a higher yield (81%) (Scheme 3, Table 4). We attributed this to the partial removal of the aliphatic benzyl protection groups on the sugar moiety with DDQ at room temperature. The NMR of (8) also required elevated temperature (140 °C) to remove rotational isomerism and simplify interpretation. Notable are the following: The carbonyl resonance at δ 198.4 in the ^{13}C NMR spectrum; Two multiplets at δ 3.05 and 2.93 in the ^{1}H NMR. These CH_2 resonances correlate with the carbon resonances at δ 38.8 and 19.7 ppm, respectively, in an edited HSQC experiment. The two multiplets also cross-couple to each other in the COSY spectrum. They represent the dihydrochalcone's CH2-CH2 moiety; the anomeric proton of the sugar moiety resonates as a doublet at δ 4.73 ppm that correlates with the anomeric carbon at δ 73.6 ppm in the edited HSQC. The J = 9.8 Hz coupling constant indicates β-stereochemistry for the *C*-glycosidic bond; The four benzylic CH_2 resonances in the δ 4.55 to 4.90 ppm range of the ^{1}H NMR spectrum indicate that the four benzyl groups on the sugar moiety remained intact (stable to the oxidation conditions); The same treatment of **(7)** with DDQ at room temperature under the conditions optimized for (5) yielded the expected dihydrochalcone-*C*-glycoside (8) in a 44% yield. Upon lowering the reaction temperature to 0 °C, the reaction proceeded slower but produced a higher yield (81%) (Scheme 3, Table 4). We attributed this to the partial removal of the aliphatic benzyl protection groups on the sugar moiety with DDQ at room temperature.

4. Conclusions

Thus the present protocol represents the first synthesis of methyl-benzyl-protected aspalathin analog (8). However, attempts to deprotect the analog (8) using BBr_3 could not lead to the successful synthesis of aspalathin. Efforts for demethylation by using the excess of BBr_3 lead to a breakdown of sugar moieties and decomposition.

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Conflicts of Interest

The authors declare no conflict of interest.

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