Hemisynthesis and spectroscopic characterization of three glycosylated 4-hydrocylonchocarpins from *Dorstenia barteri* Bureau

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Dedicated to Professor Torbjorn Norin on the occasion of his 70th birthday

Abstract

The hemisynthesis of β-galactopyranosides **2**, **3** and β-*D*-glucosaminide **4** from readily available and natural starting material, 4-hydroxylonchocarpin (**1**), has been achieved. 4-Hydroxylonchocarpin (**1**) is the major constituent of the herbaceous plant *D. barteri*. The synthetic pathway employed in this work involved the catalytic glycosylation of **1** under phase transfer catalysis (PTC) with different glycosyl donors under various reaction conditions. All these compounds were obtained using chromatographic methods. The structural elucidation of synthetic compounds was done by mass spectrometry and NMR analysis. The hydrogen-bonded phenolic hydroxyl group of **1** was not glycosylated under the employed conditions. A key feature of this reaction is the formation of pyran ring C in the product, β-galactopyranoside (**3**), and the site specific glycosylation at position C-4 of ring B. The results also confirm the isomerism equilibrium between chalcone **2** and its flavanone analog **3** which also existed in the natural sources via enzymatic reaction. This is the first report of the glycosylation of natural 4-hydroxylonchocarpin (**1**) using phase transfer catalysis.

Keywords: *Dorstenia barteri* Bureau, Moraceae, 4-hydroxylonchocarpin, flavonoid glucosides, glycosylation reaction

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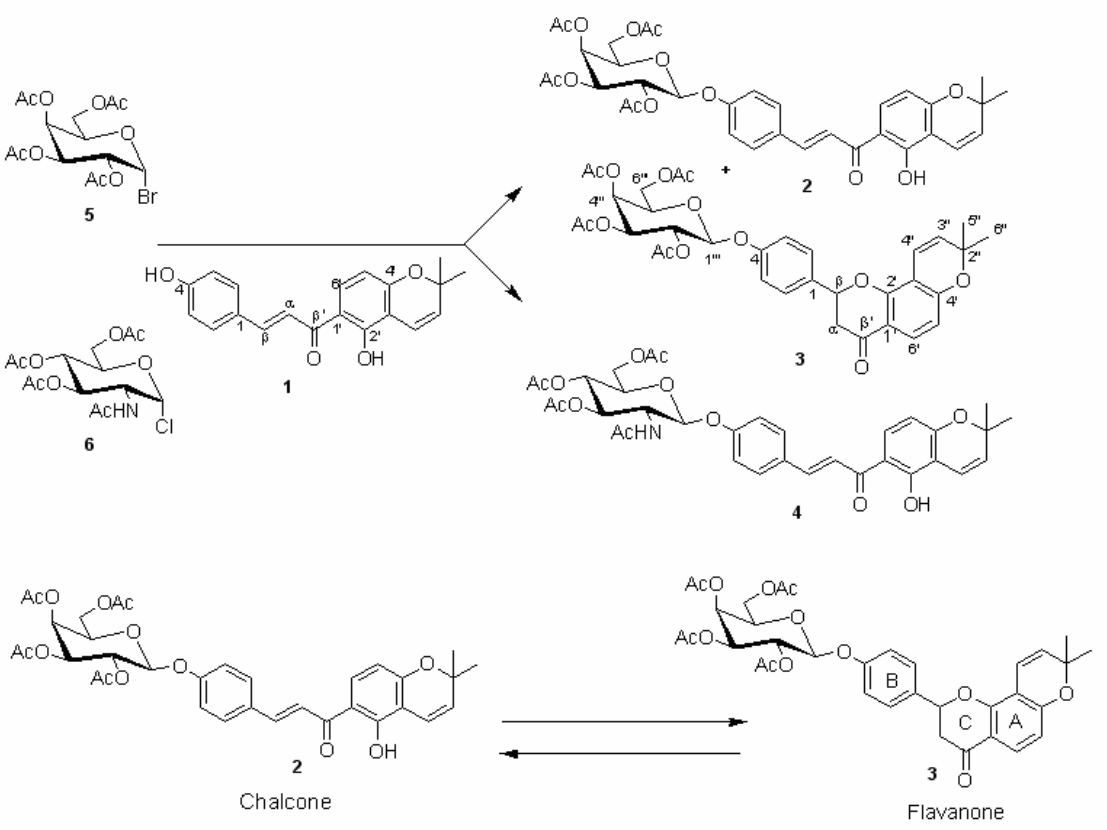
Introduction

Previous studies have suggested that certain prenylated chalcones isolated from *D. barteri* Bureau have inhibitory and chemopreventive properties against matrix metalloproteinase (MMP)-2 secretion from brain tumor-derived U87 glioblastoma cell growth.[1] Recently, our key starting material i.e. the natural product: 4-hydroxylonchocarpin (**1**) was shown to have *in vitro* activity against *P. falciparum*.[2] It is also observed that the α, β-conjugated double bond, the hydroxyl groups at $C_{2'}$, and $C_{4'}$ in ring A, and at C_4 of ring B, appear to be important for enhanced activity. However, little is known about the roles and influence of substituents at other positions. Furthermore, the presence of sugar moieties at specific positions of flavonoids may play an important role in conferring diverse biological properties to natural products. It is possible that different positions of sugar moieties on the same aglycone may allow each isomer to define the molecular recognition of its cellular target and to manifest specific biological activity. These sugars can be linked to the corresponding aglycones by glycosyltransferases, which are generally sugar-aglycone-site specific.[3,4,5] It is anticipated that glycosylated brain tumor inhibitors might cross the blood barrier more readily.[4]

As part of a program to screen derivatives of major metabolites isolated from *Dorstenia* species, we undertook the glycosylation of 4-hydroxylonchocarpin (**1**) with different glycosyl donors under various reaction conditions. The aim of the current synthetic study was to provide easy access to glycosylated flavonoids at C-4 with saturation and unsaturation at the α- and β-positions and also in the chroman part of flavanone **3** in order to assess the potential pharmocokinetic properties of the resulting synthetic products. In the present study, we describe the results of glycosylation of natural product 4-hydroxylonchocarpin (**1**) with various sugars using phase transfer catalysis (PTC).[6,7]

Table 1. Summary of Glycosylation reactions conditions of 4-hydroxylonchocarpin (**1**)

Substrate	Glycosyl donors	Reactions conditions	Product	Yield (%)
1	**5**	Bu_4NBr, aq. Na_2CO_3, $CHCl_3$, 60 ^oC, 7h	**2**	20
			3	30
		Bu_4NHSO_4, aq. Na_2CO_3, EtOAc, r.t., 18h	**2**	43
1	**6**	Bu_4NHSO_4, aq. Na_2CO_3, EtOAc, r.t., 18h	**4**	61
		K_2CO_3, dry acetone, 2h at 40 ^oC, rt., 16h	**4**	86
1	**5**	K_2CO_3, dry acetone, 2h at 40 ^oC, rt., 16h	**2**	12
			3	18



Scheme 1. Glycosylation of 4-hydroxylonchocarpin (**1**).

Results and Discussion

4-Hydroxylonchocarpin (**1**) is an abundant metabolite of several species of the genus *Dorstenia*.[11,12] The work reported here is based on this metabolite obtained from the twigs of *D. barteri* Bureau.[1] The conversion of **1** to derivatives **2**, **3,** and **4** under varied aqueous phase basic-catalyzed conditions[6,7] is shown in Scheme 1. Glycosylation of **1** with peracetylated α-*D*-galactopyranosyl bromide (**5**) under phase transfer catalysis (PTC) (Bu_4NBr, aq. Na_2CO_3, $CHCl_3$, 60 ^oC, 7h) afforded the corresponding β-galactopyranoside (**2**) and the cyclized product β-galactopyranoside (**3**) (Scheme 1).[6,7] The two compounds (**2** and **3**) are, in fact, inter-convertible, through a chalcone-flavanone equilibrium as shown in Scheme 1. This mixture is also known to exist in nature via enzymatic processes.[8,9] The solvent and temperature of the reaction were varied in order to optimize yields and to avoid the intramolecular 1,4-conjugate addition (conversion of chalcone **2** to flavanone **3**, as was described previously).[10] Surprisingly, under these conditions (Bu_4NHSO_4, aq. Na_2CO_3, EtOAc, r.t., 18h),[6] only the corresponding galactopyranoside **2** was obtained. Similar glycosylation of **1** with 2-acetamido-2-deoxy- α-D-glucopyranosyl chloride (**6**) afforded the corresponding β-D-glucosaminide (**4**).[7] In both cases, the glycosylated products were obtained in moderate yields (Table 1). It was expected that the

phase transfer of the phenolic dianions in the organic phase would be difficult under the above PTC conditions, thus explaining the low yields. The one phase, non-aqueous glycosylation reaction under dry basic conditions (K_2CO_3, dry acetone, 40 ^oC) was therefore next attempted. In the case of the glycosylation with **6**, the corresponding glycopyranoside **4** was obtained in good yield, while with galactopyranosyl bromide donor **5**, only moderate yields were obtained for the corresponding galactopyranosides **2** and **3**. Because of the limited availability of the starting material, no other methods were attempted.

There are no glycosylation studies on natural 4-hydroxylonchocarpin (**1**) reported in the literature. The glycosylated products **2**, **3**, and **4** were isolated here for the first time via hemisynthesis by transformation of natural product **1** as a starting material. The structural identities of analogs **2**, **3**, and **4** were fully established by analysis of spectral data, mainly mass, ^{1}H- and ^{13}C-NMR (Tables 2 and 3).

Table 2. ^{1}H-NMR (300 MHz) spectroscopic data of compounds[a] **1**-**4** in $CDCl_3$

Proton	**1**	**2**	**3**	**4**
2	7.55 (d, 8.4)	7.61 (d, 8.8)	7.42 (d, 8.8)	7.56 (d, 8.4)
3	6.88 (d, 8.4)	7.04 (d, 8.8)	7.06 (d, 8.4)	7.01 (d, 8.8)
5	6.88 (d, 8.4)	7.04 (d, 8.8)	7.06 (d, 8.4)	7.01 (d, 8.8)
6	7.55 (d, 8.4)	7.61 (d, 8.8)	7.42 (d, 8.8)	7.56 (d, 8.4)
α	7.43 (d, 15.4)	7.47 (d, 15.4)	2.84 (dd, 2.9, 16.5)	7.43 (d, 15.4)
			3.01 (dd, 13.8,	
β	7.83 (d, 15.4)	7.85 (d, 15.8)	16.5)	7.81 (d, 15.4)
5'	6.38 (d, 8.8)	6.39 (d, 8.8)	5.43 (dd, 2.9, 13.8)	6.38 (d, 8.8)
6'	7.71 (d, 8.8)	7.71 (d, 9.2)	6.51 (d, 8.4)	7.69 (d, 9.2)
2"	----	----	7.75 (d, 8.8)	----
3"	5.59 (d, 10.3)	5.60 (d, 9.9)	----	5.59 (d, 10.3)
4"	6.75 (d, 10.3)	6.75 (d, 10.3)	5.58 (d, 9.9)	6.74 (d, 10.3)
5" &	1.47 (s,	1.48 (s, 2xCH_3)	6.62 (d, 9.9)	1.47 (s, 2xCH3)
6"	2xCH_3)	----	1.46 (s), 1.48(s)	----
4-OH	5.24 (s)	13.70 (s)	----	13.70 (s)
2'-OH	13.79 (s)	5.13 (d, 7.7)	----	5.36 (d, 8.4)
1'''	----	5.52 (dd, 8.1, 10.6)	5.08 (d, 7.7)	4.14-4.19 (m)
2'''	----	5.15 (dd, 3.3, 10.6)	5.52 (dd, 8.1, 10.3)	5.44 (d, 9.2)
3'''	----	5.48 (d, 4.4)	5.12 (dd, 3.7, 10.6)	5.15 (d, 9.5)
4'''	----	4.11-4.18 (m)	5.48 (d, 3.7)	3.89-3.96 (m)
5'''	----	4.20-4.26 (m)	4.07-4.16 (m)	4.14-4.19 (m)
6'''	----		4.16-4.29 (m)	4.30 (dd, 5.5, 12.1)
		----		5.87 (d, 8.8)
NH	----	2.04 (s) 2.09 (s, 2x	----	2.06 (s), 2.07 (s), 2.08
OAc	----	CH_3),2.20 (s)	2.04 (s), 2.08 (s)	(s)
			2.09 (s), 2.21 (s)	1.96 (s)
NAc	----			

[a]Chemical shifts are given in ppm; multiplicities and coupling constant J (parentheses) in Hz.

Table 3. ^{13}C-NMR (75 MHz) spectroscopic data of compounds[a] **1-4** in $CDCl_3$

Carbon	**1**	**2**	**3**	**4**
1	128.5 (s)	128.5 (s)	134.3 (s)	128.5 (s)
2	130.9 (d)	130.5 (d)	127.9 (d)	130.5 (d)
3	116.4 (d)	117.5 (d)	117.5 (d)	117.4 (d)
4	158.5 (s)	158.9 (s)	157.4 (s)	159.1 (s)
5	116.4 (d)	117.5 (d)	117.5 (d)	117.4 (d)
6	130.9 (d)	130.5 (d)	129.9 (d)	130.5 (d)
α	118.3 (d)	119.4 (d)	44.6 (t)	119.4 (d)
β	144.5 (d)	143.7 (d)	79.6 (t)	143.8 (d)
β'	192.5 (s)	192.2 (s)	190.9 (s)	192.2 (s)
1'	110.1 (s)	109.8 (s)	111.6 (s)	109.8 (s)
2'	160.1 (s)	160.3 (s)	158.1 (s)	160.3 (s)
3'	114.3 (d)	114.4 (d)	115.1 (d)	114.4 (d)
4'		161.4(s)	160.1 (s)	161.3 (s)
5'		108.7 (s)	109.8 (s)	108.7 (s)
6'		130.9 (d)	129.3 (d)	130.9 (d)
2"		78.3 (s)	77.8 (s)	78.2 (s)
3"		130.4 (d)	128.3 (d)	130.1 (d)
4"		116.2 (d)	116.2 (d)	116.2 (d)
5"&6"		28.8 (q)	28.8 (q), 28.5 (q)	28.7 (q)
1'"		99.4 (d)	99.9 (d)	98.6 (d)
2'"		68.9 (d)	68.9 (d)	55.0 (d)
3'"		71.1 (d)	71.2 (d)	72.3 (d)
4'"		67.2 (d)	67.2 (d)	68.9 (d)
5'"		71.6 (d)	71.5 (d)	72.4 (d)
6'"		61.7 (t)	61.7 (t)	62.5 (t)
O<u>CO</u>CH_3,		170.7 (q), 170.6 (q)	170.7 (q), 170.6 (q)	171.2 (q), 170.9 (q)
N<u>CO</u>CH_3		170.5 (q), 169.5 (q)	170.5 (q), 169.8 (q)	170.9 (q), 169.8 (q)
OCO<u>C</u>H_3		21.1 (q), 21.1 (q)	21.1 (q), 21.0 (q)	23.7 (q), 21.1 (q)
NCO<u>C</u>H_3		21.0 (q), 20.9 (q)	21.1 (q), 21.1 (q)	21.1 (q), 20.9 (q)

Conclusions

In order to assess the importance of the double bond and the hydroxyl groups of 4-hydroxylonchocarpin (**1**) on its pharmacokinetic properties, a convenient methodology of glycosylation was used for the preparation of three flavonoid glycosides, namely, β-galactopyranosides **2** and **3**, and α-*D*-glucosaminide (**4**). This study revealed for, the first time,

formation of a non-enzymatic equilibrium between chalcone **2** and its flavanone analog **3** (Scheme 1), whose natural occurrence has been demonstrated previously.[8,9,10] Interestingly, this synthesis showed that a chelated hydroxyl group at C-2' of 4-hydroxylonchocarpin (**1**), did not undergo glycosylation in any case when both a glycosyl donors and reaction conditions are changed. Specifically, only the non-chelated phenolic hydroxyl group at C-4 of **1** afforded glycosylated reaction adducts. It will be necessary further to verify the formation of 2'-glycosylated chalcones under drastic conditions. We are currently endeavouring to optimize the yields of these glycosylated synthetic compounds and evaluating their pharmacokinetic activities in comparison to the natural product **1**. Also, the studies on the prenylated and glycosylated chalcones are currently under investigation in our laboratory.

Experimental Section

General Procedures. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. NMR spectra were recorded on a Bruker AMX-500 spectrometer or Bruker AV-300 spectrometers. ESI-MS spectra were recorded on Kratos Concepts IIH mass spectrometer. Precoated silica gel plates (Merck, Kieselgel 60 F-254, 0.5 mm) were used for PTLC. Thin layer chromatography (TLC) was performed on silica gel F_{254} (Merck) precoated aluminium sheets and spots were visualized under UV and by spraying with molybdenum solution and heating.

Glycosylation of 4-hydroxylonchocarpin (1)

Method 1. To a solution of the peracetylated α-D-galactopyranosyl bromide (**5)** (82 mg, 0.2 mmol) in $CHCl_3$ (5 mL) were added Bu_4NBr (64.4 mg, 0.2 mmol), 4-hydroxylonchocarpin (**1**) (32.2 mg, 0.1 mmol) and 0.5 M aq. Na_2CO_3 solution (1 ml). The reaction mixture was heated at 60 ^oC. The progress of the reaction was monitored by TLC. After 7 hours the reaction mixture was diluted with $CHCl_3$ and washed with saturated aq. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure and the residue was purified by flash column chromatography on silica gel, eluting with solvent mixture of increasing polarity, (90:10 to 30:70, Hexane- EtOAc) to afford recovered **1** (4 mg, 0.012 mmol), β-galactoside **2** (11 mg, 0.017 mmol, 20 %), and β-galactoside **3** (17 mg, 0.026 mmol, 30 %).

4–hydroxylonchocarpin or (*E*)-1-(5-hydroxy-2,2-dimethyl-2H-1-benzopyran-6-yl)-3-(4-hydroxyphenyl)-prop-2-en-1-one (1).[11,12,13] Orange powder, mp 203-204 °C (lit. 203-205 °C),[13] ^{1}H-NMR ($CDCl_3$, 300 MHz) see Table.2, ^{13}C-NMR ($CDCl_3$, 75 MHz) see Table.3. ESI-MS *m/z* (rel. int.):323 $[M + H]^+$ (100, calcd. For $C_{20}H_{18}O_4$, 322.3538), Elemental analysis: Calcd for $C_{20}H_{18}O_4$: C,74.52; H,5.63. Found: C,74.14; H,5.66.

Glycoside 2 or (E)-(+)-1-(5-hydroxy-2,2-dimethyl-2H-1-benzopyran-6-yl)-3-(4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-phenyl)-prop-2-en-1-one (2). Amorphous solid, $[\alpha]_D$ + 16.4 º (*c* = 1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) see Table.2, ^{13}C-NMR ($CDCl_3$, 75 MHz) see Table.3; ESI-MS *m/z* (rel. int.): 653 $[M + H]^+$ (100, calcd. for $C_{34}H_{36}O_{13}$, 652.6404), 331

$[C_{14}H_{19}O_9]^+$ (17), 323 (10), 282 (3), 242 (3), Elemental analysis: Calcd for $C_{34}H_{36}O_{13}$: C,62.57; H,5.56. Found: C,62.63; H,5.57.

Glycoside 3 or (+)-2,3-dihydro-2-(4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-phenyl)-8,8-dimethyl-4H,8H-benzo[1,2-b:3,4-b']-dipyran-4-one (3). Amorphous solid, $[\alpha]_D$ +7.6 º (*c* = 1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) see Table 2, ^{13}C NMR ($CDCl_3$, 75 MHz) see Table.3; ESI-MS *m/z* (rel. int.): 653 $[M + H]^+$ (90, calcd. for $C_{34}H_{36}O_{13}$, 652.6404), 593 (4), 332 (12), 331 $[C_{14}H_{19}O_9]^+$ (100), 323 (3), Elemental analysis: Calcd for $C_{34}H_{36}O_{13}$: C,62.57; H,5.56. Found: C,62.64; H,5.54.

Method 2. To a reaction mixture of the 2-acetamido-2-deoxy-α-D-glucopyranosyl chloride[7] (**6**) (43.8 mg, 0.12 mmol) and Bu_4NHSO_4 (33.9 mg, 0.1 mmol) in EtOAc (1 mL) were added 4-hydroxylonchocarpin (**1**) (32.2 mg, 0.1 mmol) and 1.0 M aq.Na_2CO_3 solution (1 ml). The reaction mixture was vigorously stirred at r. t. The progress of the reaction was monitored by TLC. After 18 hours the reaction mixture was diluted with EtOAc and washed with saturated aq. $NaHCO_3$ and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure and and the residue was purified by flash column chromatography on silica gel with a gradient of Hexane- EtOAc (90:10 to 40:60) as eluent to give **4** (40 mg, 0.061 mmol, 61 %).

Glycoside 4 or (E)-(-)-1-(5-hydroxy-2,2-dimethyl-2H-1-benzopyran-6-yl)-3-(4-*O*-(3,4,6-tri-*O*-acetyl-2- acetylamino-2-deoxy-β-D-glucopyranosyl)-phenyl)-prop-2-en-1-one (4). Amorphous solid, $[\alpha]_D$ – 4.7 º (*c* = 1, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) see Table 2, ^{13}C NMR ($CDCl_3$, 75 MHz) see Table 3; ESI-MS *m/z* (rel. int.): 652 $[M + H]^+$ (39, calcd. for $C_{34}H_{37}O_{12}N$, 651.6556), 332 (8), 331 (15), 330 $[C_{14}H_{20}O_8N]^+$ (100), 323 (23), 270 (7), 210 (6), Elemental analysis: Calcd for $C_{34}H_{37}O_{12}N$: C,62.67; H,5.72; N,2.15. Found: C,62.29; H,5.75; N, 2.07.

Method 3. The solution of 2-acetamido-2-deoxy-α-*D*-glucopyranosyl chloride (**6**) (43.8 mg, 0.12 mmol) in dry acetone was added dropwise to a stirred mixture of 4-hydroxylonchocarpin (**1**) (32.2 mg, 0.1 mmol) and K_2CO_3 (16.5 mg, 0.12 mmol) in dry acetone at 40 ^{o}C. The reaction mixture was kept for 2 h at 40 ^{o}C and then for 16 h at r. t. Upon completion of reaction, the reaction mixture was diluted with EtOAc and washed with 1% aq. NaOH and brine. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure and the residue was purified by flash column chromatography on silica gel, eluting with solvent mixture of increasing polarity, (90:10 to 40:60, Hexane- EtOAc) to provide **4** (56 mg, 0.086 mmol, 86 %).

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