



# Isolation of a new disaccharide nucleoside from *Helleborus caucasicus*: structure elucidation and total synthesis of hellecaucaside A and its $\beta$ -anomer



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## ABSTRACT

Hellecaucaside A, a new disaccharide nucleoside featuring a 2'-O- $\alpha$ -D-ribofuranosyluridine skeleton and a 4-hydroxybenzoyl group at the 5' position, was isolated from the underground part of *Helleborus caucasicus*. The structure of the compound was elucidated by means of chemical degradation and spectroscopic analyses, such as 1D/2D NMR, chiral-GC, and HRMS. The total synthesis of hellecaucaside A and its  $\beta$ -anomer was accomplished, unequivocally confirming the structure of the natural product.

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## 1. Introduction

The genus *Helleborus* includes around 25 species of evergreen flowering plants belonging to the Ranunculaceae family. Native mainly to Europe and Asia, some of these plants have been screened for their biological and medicinal potential and showed interesting properties such as insecticidal,<sup>1</sup> antianalgesic, antirheumatic,<sup>2</sup> immunosuppressive,<sup>3</sup> and anticancer activities.<sup>4</sup> *Helleborus caucasicus*, a plant endemic to Greece, Turkey, and Caucasus, is growing wild in the west part of Georgia. The MeOH extracts from this plant have recently attracted attention because of their high cytotoxicity toward human lung carcinoma (A549) and colorectal adenocarcinoma (DLD-1) cell lines.<sup>5</sup> The chemical constituents of *H. caucasicus* have been studied, allowing the isolation of secondary metabolites such as polyhydroxylated and polyunsaturated furostanol glycosides, bufadienolides, and ecdysteroids.<sup>6,7</sup> The biological activities of these metabolites have not been evaluated so far.

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In continuation with our efforts to discover new bioactive chemical entities from vegetal species,<sup>8</sup> we have been interested in studying the chemical composition of the MeOH extracts from the underground part of *H. caucasicus*. A new disaccharide nucleoside featuring a 2'-O- $\alpha$ -D-ribofuranosyluridine skeleton, hellecaucaside A (**1**), was isolated. The compound was identified on the basis of chemical degradation and spectroscopic analyses, including 1D and 2D NMR experiments, chiral GC, and HRMS analyses.

Disaccharide nucleosides are a unique family of natural compounds exhibiting diverse biological properties such as antibacterial, antiviral, anticancer, insecticidal, and fungicidal activities.<sup>9–11</sup> According to the structure of the sugar chain, disaccharide nucleosides are subdivided into three main groups: (1) nucleosides with two hexopyranose residues; (2) nucleosides with a ribofuranose and an additional hexopyranose unit; (3) nucleosides with two ribofuranose residues.<sup>11</sup> Disaccharide nucleosides in which the two ribofuranose units are linked together via an O-glycosidic bond (3rd group), such as hellecaucaside A (**1**), have been mainly found in eukaryotic tRNAs and poly(ADP-ribose).<sup>11</sup> In tRNAs, disaccharide nucleosides feature a  $\beta$ -glycosidic linkage (1,2-*trans*) coming from the enzymatic post-translational ribosylation of adenosine or guanosine residues.<sup>12</sup> In contrast, disaccharide nucleosides with

the  $\alpha$ -configuration (1,2-*cis*) between ribose units have been isolated from the degradation products of poly(ADP-ribose), which is a post-translational modification of proteins in eukaryotic cells.<sup>13</sup> For instance, enzymatic hydrolysis of poly(ADP-ribose) provided 2'-*O*- $\alpha$ -D-ribofuranosyladenosine,<sup>14</sup> a nucleoside structurally related to hellecaucaside A (**1**).

The important biological properties of 2'-*O*- $\alpha$ -D-ribofuranosyl-nucleosides and their derivatives prompted us to undertake the synthesis of hellecaucaside A (**1**) and its biologically relevant  $\beta$ -anomer **17**. Our motivations were threefold: (1) to unequivocally confirm the structure of **1**; (2) to obtain larger quantities of **1**, allowing its screening for diverse biological/medicinal properties; (3) to pave the way for the synthesis of derivatives of **1** with diverse substituents at the 5' position. Herein, we report the isolation, structural elucidation, conformational analysis, and total synthesis of hellecaucaside A (**1**) and its  $\beta$ -anomer **17**.

## 2. Results and discussion

### 2.1. Isolation of hellecaucaside A (**1**)

Dry roots and rhizomes of *H. caucasicus* were defatted by hexanes extraction. The dry residue was successively extracted, first with hot MeOH and then with 80:20 MeOH–H<sub>2</sub>O. The extracts were filtrated and pooled. After evaporation of the MeOH, the aqueous phase was extracted with EtOAc. Further purification of the organic fraction using a combination of different chromatographic techniques, such as silica gel, Diaion<sup>®</sup> resin, and semi-preparative reversed-phase C<sub>18</sub> HPLC, yielded pure **1**.

Hellecaucaside A (**1**) was obtained as a white amorphous solid and its molecular formula determined to be C<sub>21</sub>H<sub>24</sub>N<sub>2</sub>O<sub>12</sub> on the basis of its HRESIMS pseudomolecular ion peak at *m/z* 519.1248 [M+Na]<sup>+</sup>. Analysis of <sup>13</sup>C and DEPT-135 NMR spectra revealed the presence of two sp<sup>3</sup> oxygenated methylenes, eight sp<sup>3</sup> oxymethines, six sp<sup>2</sup> methines, and five sp<sup>2</sup> quaternary carbons (Table 1). Examination of the 2D <sup>1</sup>H–<sup>1</sup>H COSY spectrum allowed the identification of four spin systems that were assigned to a *p*-hydroxybenzoyl group at  $\delta_{\text{H}}$  7.91 (d, 2H, *J* 8.8 Hz, H-2'', H-6'') and 6.86

(d, 2H, *J* 8.8 Hz, H-3'', H-5''), two pentose sugar units with their anomeric protons at  $\delta_{\text{H}}$  5.89 (d, 1H, *J*<sub>1',2'</sub> 2.4 Hz, H-1') and 5.36 (d, 1H, *J*<sub>1'',2''</sub> 4.2 Hz, H-1''), and a *cis*-olefinic bond with protons at  $\delta_{\text{H}}$  7.72 (d, 1H, *J*<sub>6,5</sub> 8.1 Hz, H-6) and 5.48 (d, 1H, *J*<sub>5,6</sub> 8.1 Hz, H-5). The latter showed long-range <sup>1</sup>H–<sup>13</sup>C correlations with carbons at  $\delta_{\text{C}}$  166.12 (C-4) and 151.99 (C-2), suggesting the presence of a uracil moiety (Fig. 1).<sup>15</sup> The connectivity between these groups was established by HMBC cross-peaks from H-1' to  $\delta_{\text{C}}$  141.68 (C-6) and C-2, from H-1'' to 78.61 (C-2'), and from  $\delta_{\text{H}}$  4.70 (dd, 1H, *J*<sub>5'a,5'b</sub> 12.5, *J*<sub>5'a,4'</sub> 2.7 Hz, H-5'a) and 4.52 (dd, 1H, *J*<sub>5'b,5'a</sub> 12.5, *J*<sub>5'b,4'</sub> 4.0 Hz, H-5'b) to  $\delta_{\text{C}}$  167.63 (C-7''). Also, NOESY cross-peaks were observed between H-6 and H-1' and between H-1'', H-1', and  $\delta_{\text{H}}$  4.46 (dd, 1H, *J*<sub>2',3'</sub> 5.2, *J*<sub>2',1'</sub> 2.4 Hz, H-2'), which supported the connection pattern (Fig. 1). Both sugar moieties were determined to be D-ribose by acid hydrolysis followed by chiral GC and comparison with an authentic standard (see Supporting information). Furthermore, the uracil moiety was formally identified by HPLC–UV–MS from the same reaction mixture (see Supporting information). The first ribose unit was determined to be a  $\beta$ -anomer from NOESY correlations between H-6 and all other sugar protons except H-4'. The second ribose unit was determined to be an  $\alpha$ -anomer from the coupling constant of 4.2 Hz, which is almost the same as that of a methyl  $\alpha$ -D-ribofuranoside.<sup>16</sup> On the basis of the above chemical and spectroscopic evidences, the structure of compound **1** was established as 1-[5-(4-hydroxybenzoyl)-2-*O*- $\alpha$ -D-ribofuranosyl]- $\beta$ -D-ribofuranosyl]uracil, and named hellecaucaside A (Fig. 1).

### 2.2. Conformational analysis of **1** and **2**

Hellecaucaside A (**1**) was subjected to methanolysis under Zemplén conditions (catalytic NaOMe in MeOH) to give disaccharide nucleoside **2** (Scheme 1). Coupling constants for both of the ribofuranose residues (A and B) in **1** and **2** were compared with those of synthetic 2'-*O*- $\alpha$ -D-ribofuranosyladenosine.<sup>17,18</sup> As shown in Table 2, the coupling constants of ribofuranose A in **1** and **2** were slightly different. Therefore, a conformational analysis of the ribofuranose rings was undertaken for the three molecules. In order to do so, the coupling constants measured by NMR were entered into the Matlab program developed by Hendrickx.<sup>19</sup> The program

**Table 1**  
<sup>1</sup>H and <sup>13</sup>C NMR data for disaccharide nucleosides **1** and **17** in CD<sub>3</sub>OD<sup>a</sup>

Position	<b>1</b>		<b>17</b>	
	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{c}}$	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{c}}$
2	151.99 (s)		152.22 (s)	
4	166.12 (s)		166.04 (s)	
5	102.65 (d)	5.48 (d, 1H, 8.1)	102.89 (d)	5.54 (d, 1H, 8.0)
6	141.68 (d)	7.72 (d, 1H, 8.1)	142.84 (d)	7.67 (d, 1H, 8.1)
1'	90.45 (d)	5.89 (d, 1H, 2.4)	91.24 (d)	5.96 (d, 1H, 2.7)
2'	78.61 (d)	4.46 (dd, 1H, 5.2, 2.4)	79.83 (d)	4.40 (m, 1H, m)
3'	70.55 (d)	4.30 (dd, 1H, 7.5, 5.2)	70.54 (d)	4.39 (m, 1H, m)
4'	83.06 (d)	4.35 (ddd, 1H, 7.6, 3.8, 2.6)	82.93 (d)	4.20 (m, 1H, m)
5'a	63.92 (t)	4.70 (dd, 1H, 12.5, 2.7)	64.28 (t)	4.63 (dd, 1H, 12.5, 2.9)
5'b		4.52 (dd, 1H, 12.5, 4.0)		4.46 (dd, 1H, 12.4, 4.8)
1''	102.00 (d)	5.36 (d, 1H, 4.2)	109.02 (d)	5.12 (s, 1H, s)
2''	73.84 (d)	4.09 (dd, 1H, 6.2, 4.2)	76.28 (d)	4.07 (d, 1H, 4.6)
3''	71.79 (d)	4.01 (dd, 1H, 6.1, 2.2)	71.82 (d)	4.17 (dd, 1H, 7.4, 4.6)
4''	87.75 (d)	4.12 (m, 1H, m)	85.07 (d)	3.94 (ddd, 1H, 7.6, 5.5, 3.1)
5''a	63.32 (t)	3.63 (dd, 1H, 12.1, 3.7)	63.79 (t)	3.74 (dd, 1H, 12.2, 3.2)
5''b		3.59 (dd, 1H, 12.1, 4.1)		3.56 (dd, 1H, 12.1, 5.6)
1'''	121.79 (s)		121.74 (s)	
2''', 6'''	132.98 (d)	7.91 (d, 2H, 8.8)	132.96 (d)	
3''', 5'''	116.43 (d)	6.86 (d, 2H, 8.8)	116.34 (d)	
4'''	163.94 (s)		163.85 (s)	
7'''	167.63 (s)		167.65 (s)	

<sup>a</sup> NMR spectrum obtained at 400 MHz for <sup>1</sup>H and 100 MHz for <sup>13</sup>C.

<sup>b</sup> Multiplicity deduced from DEPT-135 spectrum.

<sup>c</sup> d: doublet, dd: doublet of doublet, ddd: doublet of doublet of doublet, m: multiplet, s: singlet, t: triplet.

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