

**The Vitamin C Analogue 2-O- $\beta$ -D-Glucopyranosyl-L-ascorbic Acid in Rhizomes, Stems and Leaves of *Lycium barbarum***

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**Abstract:** Awareness of health benefits of goji berries coming from their bioactive compounds, mostly antioxidants like ascorbic acid, has grown. Recently, an ascorbic acid analogue from goji berries, the 2-O- $\beta$ -D-glucopyranosyl-L-ascorbic acid has been reported. In rats, the analogue is absorbed intact and in the form of free vitamin C and consequently has been proposed as a provitamin C. Synthesis of the analogue is demanding and laborious and therefore reliable natural sources are searched. Knowledge concerning the analogue's occurrence in other parts of goji plant is lacking. The aim of this study was to evaluate the contents of 2-O- $\beta$ -D-glucopyranosyl-L-ascorbic acid in rhizomes, stems and leaves from *Lycium barbarum*. Rhizomes, stems and leaves were extracted and the content of 2-O- $\beta$ -D-glucopyranosyl-L-ascorbic acid and non glucosylated, free ascorbic acid was determined by HPLC-DAD. 2-O- $\beta$ -D-Glucopyranosyl-L-ascorbic acid was found in all goji plant tissues investigated. Based on dry weight, 3.34 mg/100 g were found in the leaves, 4.05 mg/100 g in the stems and up to 12.6 mg/100 g in the rhizomes. Nevertheless, the analogue content in goji berries is much higher (40 to 280 mg/100 g dry weight). The present study confirmed the presence of 2-O- $\beta$ -D-glucopyranosyl-L-ascorbic acid in rhizomes, stems and leaves of *Lycium barbarum*. However, their content compared to goji berries is considerably lower.

**Keywords:** Ascorbic acid · Ascorbic acid analogue · 2-O- $\beta$ -D-Glucopyranosyl-L-ascorbic · Goji · *Lycium barbarum*

**1. Introduction**

Goji berries (*Lycium spp.*), originated in China and have received a considerable attention due to the content of bioactive compounds.<sup>[1]</sup> Goji berries have been used in traditional Chinese medicine for centuries. Due to the increasing knowledge about the role of antioxidants and their importance in fighting free radicals, consumption of high antioxidant-containing foods increased concurrently during the last decades.

In addition to the *Lycium* berries, other parts of the plant such as rhizome, bark, stems, leaves and flowers (Fig. 1) have been studied for their antioxidant activity.<sup>[2–8]</sup> Interest in the composition has intensified due to the rising awareness of the possible health benefits provided by goji micronutrients and phytochemicals.

Beside the already identified bioactive compounds, a novel stable ascorbic acid (AA) analogue named 2-O- $\beta$ -D-glucopyranosyl-L-ascorbic acid (2- $\beta$ -gAA) has been recently



Fig. 1. Stems, leaves and a flower from goji plant.

identified as being one of the main biologically active components in goji berries.<sup>[9]</sup> The presence of 2- $\beta$ -gAA in different goji berries has been studied extensively,<sup>[8–12]</sup> whereas the knowledge concerning the analogue's occurrence in goji plant's rhizomes, stems and leaves is still lacking. According to a study by Toyoda-Ono *et al.*, the 2- $\beta$ -gAA was not found in the rhizomes or the leaves.<sup>[12]</sup> Meanwhile, Bubloz *et al.* confirmed the presence of 2- $\beta$ -gAA in berries from the *Solanacea* family.<sup>[13]</sup>

It was reported that 2- $\beta$ -gAA acted as a provitamin C since it maintained, to some extent, the level of AA in the tissues of rats.<sup>[14]</sup> The 2- $\beta$ -gAA was even proposed as a stable AA substitute for clinical applications. Unfortunately, synthetic access to this promising compound by chemical or enzymatic approach is demanding, laborious and not efficient.<sup>[12,15]</sup>

The aim of this study was to evaluate the content of 2- $\beta$ -gAA in the rhizomes, stems and leaves of goji plant.

**2. Materials and Methods****2.1 Materials**

The *Lycium barbarum* plant material (rhizomes, stems and leaves) was harvested in Veyras, Switzerland, in March 2019, the morning before analysis. Plant material was transported at room temperature in humid conditions.

**2.2 Chemicals and Reagents**

AA was obtained from Fluka Analytical (Damstadt, Germany) and sulfuric acid was acquired from Acros Organic (Geel, Belgium). Acetonitrile of HPLC grade was purchased from Macron Fine Chemicals Avantor (Ankara, Turkey) and formic acid and oxalic acid from Sigma Aldrich (Buchs, Switzerland). The

solvents DMSO, dichloromethane, ethylacetate, acetic acid and methanol were of analysis grade and from Sigma Aldrich (Buchs, Switzerland). NMR spectra were done in D<sub>2</sub>O (99.8% D), obtained from ARMAR (Döttingen/Switzerland). 5,6-Isopropylidene-L-ascorbic acid (>99%) was bought from Fluorochem (Hadfield, United Kingdom) and the cation-exchange resin, IR-120 (H<sup>+</sup>) from Merck (Darmstadt, Germany). Benzyl bromide (98%), tetra-*O*-acetyl- $\alpha$ -D-glucopyranosyl bromide (>93%), Pd/C (10%), sodium methanolate (25 wt.% in methanol) were obtained from Sigma Aldrich (Buchs, Switzerland). Deionized water was used from Milli-Q purification system (Millipore AG, Zug, Switzerland).

Oxalic acid solution: 10 g/L were solubilized in water. Oxalic acid protects the AA from oxidation but impedes 2- $\beta$ -gAA detection.

### 2.3 Sample Homogenization

First, 1 g of rhizomes and 5 g of stems and leaves, respectively, were weighted. Rhizomes and stems were mixed (Ultraturax T25, IKA, Staufen, Germany) for 2 min at 13'500 rounds/min with 10 mL of the oxalic acid solution for AA analysis or with water for 2- $\beta$ -gAA analysis. To prepare the leaves, the Ultraturax was used for 2 min at 9'500 rounds/min with 20 mL of the oxalic acid solution or water.

### 2.4 Extraction Procedure

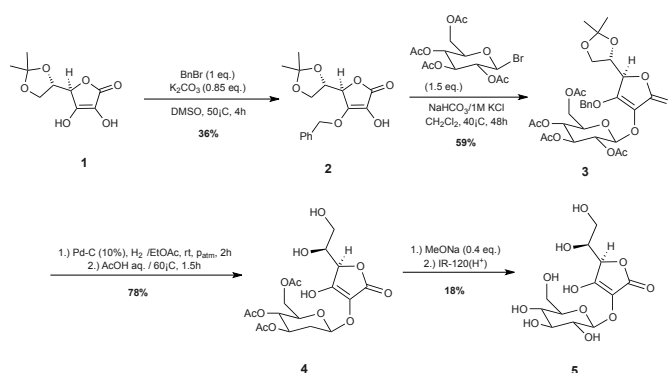
The method described by Toyoda-Ono *et al.*<sup>[12]</sup> with modifications proposed by Kosińska-Cagnazzo *et al.*<sup>[9]</sup> was used to extract 2- $\beta$ -gAA and AA. Briefly, the homogenized sample was ultrasonicated for 10 min at 35 kHz (VWR, Dietikon, Switzerland). After centrifugation for 15 min at 2800g (NUVE, Ankara, Turkey), the supernatant was collected. To the deposit, 5 mL of oxalic acid solution (10 g/L oxalic acid in water) for AA extraction, or only water (for 2- $\beta$ -gAA extraction) were added and blended with a Vortex Genius 3 (IKA, Chavannes-de-Bois, Switzerland). The plant material was extracted twice, the extracts were combined and adjusted to 20 mL. For stem analysis, 10 mL of extract were used. Again, the plant material was extracted twice, the extracts were combined and adjusted to 50 mL. After filtration (0.45  $\mu$ m nylon syringe filter CHROMAFIL®, Machery-Nagel, Düren, Germany), the extracts were directly analyzed by HPLC.

### 2.5 Synthesis of 2-*O*- $\beta$ -D-Glucopyranosyl-L-ascorbic acid

The total synthesis of 2- $\beta$ -gAA was realized over four steps (Scheme 1). Commercially available 5,6-isopropylidene-L-ascorbic acid was selectively benzyl protected at the 3-hydroxyl position<sup>[12]</sup> (yield: 36%), followed by the glycosylation with tetra-*O*-acetyl- $\alpha$ -D-glucopyranosyl bromide at the 2-hydroxyl position (yield: 59%).<sup>[16]</sup> The generated 2-*O*-glucoside was debenzylated by hydrogenolysis in the presence of Pd/C and deisopropylidenated under acidic conditions (yield: 78%).<sup>[12]</sup> After that, the acetyl groups were removed under MeONa treatment, neutralized with a cation-exchange resin, IR-120 (H<sup>+</sup>) and recrystallized to obtain some 10 mg of 2- $\beta$ -gAA (yield: 18%).<sup>[17]</sup> To confirm identity of the synthesis product, <sup>1</sup>H and <sup>13</sup>C NMR in D<sub>2</sub>O were recorded with a Bruker 400 MHz UltraShield (broad band indirect, BBI).

### 2.6 HPLC Analysis of Ascorbic Acid and 2-*O*- $\beta$ -D-Glucopyranosyl-L-ascorbic acid

An Agilent 1220 Infinity series liquid chromatograph (Agilent Technologies, CA, USA) comprised of an auto-sampler, a binary pump and a G4294B UV-DAD detector (Agilent Technologies 110 Series, Agilent Technologies, CA, USA) was employed for the chromatographic separation as previously described.<sup>[18]</sup> Briefly, 5  $\mu$ L were analyzed with an amino column (Aminex HPX-87H Ion exclusion, 300×7.8 mm i.d., particle size 5  $\mu$ m, Bio-Rad, Hercules,



Scheme 1. Schematic presentation of the four synthesis steps. Percentage under the reaction arrow indicates the synthesis yield of the individual step.

CA, USA) equipped with a precolumn (30×4.6 mm, cation H cartridge for amino column). The mobile phase was composed of 5 mmol/L sulfuric acid and was delivered in an isocratic mode at a constant flow rate of 0.5 mL/min. The column temperature was set to 40 °C. AA and 2- $\beta$ -gAA were detected at 254 nm. The retention time was 8.6 min for AA and 11.3 min for 2- $\beta$ -gAA with a total run time of 30 min. Chromatographic peaks were identified by comparison of retention times and UV spectra with those obtained for the standard AA and the synthesis product 2- $\beta$ -gAA. AA was quantified by external calibration. Quantification of 2- $\beta$ -gAA was done traditionally with the AA calibration curve and a conversion factor reported by Tai and Godha.<sup>[19]</sup>

### 2.7 Dry Weight

Dry weight (dw) of the raw material was determined with a halogen moisture analyzer (Mettler Toledo, Greifensee, Switzerland). The temperature was set at 110 °C. All samples were analyzed in triplicate.

### 2.8 Presentation of Results

Results of dry weight, AA and 2- $\beta$ -gAA are indicated as mean  $\pm$  standard deviation from triplicate analysis.

### 3. Results and Discussion

The structure of the synthesized reference compound for the vitamin C analog has been confirmed by NMR in D<sub>2</sub>O (4.67 ppm). <sup>1</sup>H, <sup>13</sup>C, COSY, HSQC and HMBC were recorded. The <sup>1</sup>H-NMR spectrum of the 2- $\beta$ -gAA exhibited two doublets on the anomeric regions (4.88 and 4.79 ppm), one for the ascorbic acid moiety and one for the glucose unit. All the other signals consist of typical carbohydrate signals with the five exocyclic protons resonating between 3.55 and 4.00 ppm. The four remaining endocyclic protons are between 3.45 and 3.30 ppm. The <sup>13</sup>C-NMR spectrum displayed 12 resonances for three sp<sup>2</sup> hybridized carbons (173.7, 164.7 and 119.4), three oxymethine carbons (77.7, 70.3 and 63.3), and a glucose unit (103.5, 77.6, 76.7, 74.2, 70.5, and 61.7). The obtained <sup>1</sup>H and <sup>13</sup>C-NMR spectra were like those published by Toyoda-Ono *et al.*<sup>[12]</sup>

The contents of AA and 2- $\beta$ -gAA of the rhizomes, stems and leaves are summarized in the Table 1. For an easier comparison, this table gives also the analogue's content of goji berries. The 2- $\beta$ -gAA was found in all three plant tissues of the *Lycium barbarum* plant analyzed. The analogue's content ranged from 3.34 mg/100 g dw for the leaves up to 12.6 mg/100 g dw for the rhizomes with the stems containing 4.05 mg/100 g dw. In a previous study, the analogue was observed exclusively in the berries,<sup>[12]</sup> a possible consequence of the more efficient analytical equipment used in the present study. Nevertheless, the analogue content is much higher in goji berries ranging from 40 to 280 mg/100 g dw.<sup>[18]</sup> The highest relative standard deviation is

observed for the values of 2- $\beta$ -gAA in rhizomes. This is a result of the low extraction reproducibility, coming from the difficulty to break and shear the hard and resistant rhizome bark.

Table 1. Content of 2- $\beta$ -gAA of the *Lycium barbarum* rhizomes, stems and leaves, indicated in mg/100 g dw, mean  $\pm$  standard deviation, n=3.

	dw	2- $\beta$ -g-AA	AA
Rhizome	22%	12.6 $\pm$ 2.3	11.6 $\pm$ 1.2
Stem	14%	4.05 $\pm$ 0.05	3560 $\pm$ 530
Leave	14%	3.34 $\pm$ 0.33	111 $\pm$ 9.7
Berry <sup>[11]</sup>	-	35–280	

The stems and leaves showed a higher content of AA compared to 2- $\beta$ -gAA, whereas no major differences were observed for rhizomes. No studies about AA content in goji plants were available for comparison. However, as reviewed by Smirnoff and Wheeler,<sup>[20]</sup> AA can be found in all type of cells, except in dry seeds. The rhizomes showed higher 2- $\beta$ -gAA content compared to the two other tissues. The rhizomes are known to be a storage organ for starch, proteins and other nutrients.<sup>[21]</sup> For this reason, it is not surprising to find the highest amount of 2- $\beta$ -gAA in this part of the plant. However, compared to leaves and stems, the rhizomes have remarkably low contents of AA.

#### 4. Conclusions

The current study confirmed the presence of 2- $\beta$ -gAA in rhizomes, stems and leaves of *Lycium barbarum* plants. The contents are lower compared to goji berries. However, leaves could be harvested over a longer time period than the berries.

#### Conflict of interest

The authors have no conflict of interest to report.

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