

Cyanidin 3-Gentiobioside from Primary Leaves of Rye (*Secale cereale* L.)

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An anthocyanin has been isolated from primary leaves of *Secale cereale* L. and identified as cyanidin 3-O-gentiobioside on the basis of UV spectroscopy, hydrolysis and chromatography.

Introduction

Primary leaves of rye (*Secale cereale* L. cv. Kustro) show a heterogeneous, tissue-specific pattern of flavonoids being composed of the two C-glycosyl-flavone glycosides isovitexin 2''-O-arabinoside and isovitexin 2''-O-galactoside (located in the epidermal layers) [1, 2], the two flavone glycosides luteolin 7-O-diglucuronide and luteolin 7-O-diglucuronide 4'-O-glucuronide [3] and the two anthocyanins cyanidin 3-O-glucoside [2, 4] and cyanidin 3-O-diglycoside [2], both flavones and anthocyanins located in the mesophyll [2]. The latter awaited structural elucidation and we report here its isolation and identification as cyanidin 3-O-gentiobioside. This completes the structure elucidation of flavonoids from rye primary leaves and will be the basis of a study of the turnover [2] and enzymatic synthesis of the anthocyanins in rye primary leaves.

Material and Methods

Plant material and standard markers

Plant material and growing conditions have been described elsewhere [2]. Standard markers of cyanidin glycosides came from extracts of *Papaver rhoeas* (3-sophoroside), *Petunia hybrida* (3-sophoroside, 3-gentiobioside, 3-glucoside) [7, 8] and *Heliconia psittacorum* (3-rutinoside, 3-glucoside) [Busch, unpublished]. Authentic anthocyanidins were from our institute collection.

Isolation and purification of cyanidin

3-O-gentiobioside: compound **RII**

Primary leaves (5 days old) were extracted with 2% HCl in MeOH (v/v). **RII** was separated from other phenolic constituents by prep. TLC in SS2. Purification was achieved by CC on cellulose MN 2100 ff, 2.5 × 15 cm (Macherey, Nagel & Co., Düren, FRG) with H₂O as eluant.

Acid hydrolysis

For complete hydrolysis **RII** was heated in 25% aq. HCl (v/v) for 7 min at 100 °C. The aglycone was extracted into a small volume of amyl alcohol followed by TLC in SS3, SS4 and SS5. The aq. phase was kept for sugar identification (TLC in SS6). Partial hydrolyses were done with 1 N HCl by heating for 2.5, 5, 10, 20, 30, and 40 min, respectively, at 100 °C. The hydrolysates were chromatographed in SS2 and analyzed by HPLC [5].

H₂O₂ treatment

To dried **RII** was added 3 drops of 0.1 M NH₄OH followed by 2 drops of H₂O₂ [6]. After 4 h at room temperature the solution was evaporated to dryness and the residue dissolved in H₂O. This was chromatographed in SS6.

Thin-layer chromatography

On microcrystalline cellulose ("Avicel", Macherey, Nagel & Co., Düren, FRG): (SS1) HCl–H₂O (3:97), (SS2) acetic acid–HCl–H₂O (15:3:82), (SS3) *n*-butanol–acetic acid–H₂O (4:1:5, upper phase), (SS4) isopropanol–acetic acid–H₂O (1:4:5), (SS5) acetic acid–HCl–H₂O (30:3:10), by vol.; on silica gel according to Hansen [9] (Merck, Darmstadt, FRG), impregnated with a 0.5 M NaH₂PO₄ solution before use: (SS6) 0.1 M lactic acid–isopropanol–acetone (1:2:2), development of sugar spots by spraying with aniline–diphenylamine–acetone–H₃PO₄ (2:2:100:15, v/w/v/v) and heating for 15 min at 100 °C (glucose and gentiobiose gave blue colour).

Spectroscopy

UV/Vis spectral analysis was according to Harborne [7].

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Results and Discussion

Rye primary leaves accumulate two anthocyanins, cyanidin 3-O-glucoside [2, 4] as a major constituent and a cyanidin 3-O-diglycoside as a minor one [2]. This finding was based on high performance liquid chromatographic identification and controlled hydrolyses [5]. It was found that the basic structure of the diglycoside was identical with the major rye compound cyanidin 3-O-glucoside and thus both the second sugar attached and the disaccharide linkage remained to be identified. This pigment was purified by thin layer chromatography on microcrystalline cellulose followed by cellulose column chromatography.

The UV/Vis spectrum in MeOH (0.01% HCl) showed a visible max. at 523 nm with $E_{440}/E_{vis,max}$ of 30%. The addition of a few drops of 5% $AlCl_3$ (in MeOH) resulted in a bathochromic shift of 25 nm. Thus the spectral data indicated the presence of a catechol nucleus and 3-glycoside.

Partial acid hydrolysis gave cyanidin 3-O-glucoside and cyanidin, complete acid hydrolysis cyanidin and glucose. Treatment of the pigment with H_2O_2 in NH_4OH [6] resulted in the liberation of gentiobiose. These data were confirmed by co-chromatography with authentic markers (cyanidin, glucose, gentiobiose). In addition, pigments (cyanidin 3-glucoside, 3-sophoroside, 3-gentiobioside, and 3-rutinoside)

Table I. Co-chromatographic data* ($R_f \times 100$) of cyanidin 3-O-gentiobioside (rye compound **RII**) and its products from complete and partial hydrolyses. Further details in "Material and Methods".

Compound	SS1	SS2	SS3	SS4	SS5	SS6
(RII) Cyanidin						
3-gentiobioside	15	46	24	—	—	—
Cyanidin						
3-glucoside	7	23	38	—	—	—
Cyanidin	—	—	65	60	48	—
Glucose	—	—	—	—	—	40
Gentiobiose	—	—	—	—	—	28

* Co-chromatography with cyanidin 3-O-gentiobioside and 3-O-glucoside from *Petunia hybrida*, with cyanidin from complete hydrolyses of extracts from *P. hybrida*, with glucose and gentiobiose from commercial sources. **RII** differed from cyanidin 3-rutinoside (SS1: 19; SS2: 41; SS3: 37) and cyanidin 3-sophoroside (SS1: 34; SS2: 59; SS3: 29). The **RII**-aglycone (cyanidin) differed from delphinidin (SS3: 41; SS4: 29; SS5: 31), petunidin (SS3: 52; SS4: 45; SS5: 44) and peonidin (SS3: 72; SS5: 62).

isolated from other plants were used for chromatographic comparison. Table I summarizes the chromatographic data obtained.

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