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ORIGINAL PAPER

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Identification of a new flavanol glucoside from barley (*Hordeum vulgare* L.) and malt

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Abstract (2*R*,3*S*)-Catechin-7-*O*-β-D-glucopyranoside is isolated from barley (*Hordeum vulgare* L.) and malt for the first time. For identification, the glucoside was isolated from acetone-water extracts of malt by polyamide clean-up and semi-preparative HPLC. The structure was elucidated by various NMR techniques, fast atom bombardment mass spectrometry and UV and circular dichroism spectroscopy. The investigation of 20 different barley varieties and the corresponding malts by HPLC with UV and coulometric electrode array detection revealed that the amount of the glucoside increases during malting. In contrast, the content of the monomeric (+)-catechin decreases and (–)-epicatechin was not detected.

Keywords *Hordeum vulgare* · Barley · Malt (2*R*,3*S*)-Catechin-7-*O*-β-D-glucopyranoside · Flavan-3-ol glucoside · Catechin

Abbreviation used *n.d.*: not determinable

Introduction

Polyphenols are important secondary metabolites of plants and are widespread in nature. They are divided into major classes on the basis of their skeleton e.g. flavonoids. Today, more than 5000 flavonoids are known which appear in various structural classes according to the oxidation state of the central pyran ring of the C_{15} aglycon skeleton. The large number of compounds result from the different derivatisations of the diphenylpropane skeleton by hydroxylation, methoxylation, glycosylation or other modifications [1].

In foodstuffs and in the beer brewing process, polyphenols are mainly minor compounds. Nevertheless, due to their properties and reactivity they are important to brewers because they influence several stages of the brewing process and the overall beer stability e.g. formation of haze, colour, taste, filtration, foam stability and redox state [2, 3]. The proanthocyanidins and catechins of barley (*Hordeum vulgare* L.) in particular, are known to be involved in the formation of beer haze due to their affinity for proteins [4, 5, 6, 7]. The role of these compounds in beer flavour stability is less certain and is the subject of much current debate [6]. About 80% of the beer proanthocyanidins originate from malt and the rest from hops [7, 8].

The structures of two dimeric (procyanidin B3 and prodelphinidin B3) and four trimeric (procyanidin C2 and three prodelphinidins) proanthocyanidins of barley have been established by NMR spectroscopy. They consist of catechin and gallocatechin units from which catechin also occurs as a monomeric compound [7, 9, 10]. Furthermore, the presence of procyanidin B6 [11], a trimer consisting only of gallocatechin units and the monomeric gallocatechin [12], a gallocatechin dimer [13] and different dimeric proanthocyanidins in addition to the established dimers [14] have been reported on the basis of different analytical methods. In the latter two cases, HPLC with mass spectrometric detection was used. Whittle et al. [15] have investigated proanthocyanidins in barley by HPLC-ESI-MS. They have detected oligomeric flavanols up to pentamers that have mainly been only tentatively identified. Nevertheless, besides the six proanthocyanidins mentioned above, the structure of all other compounds have so far not been confirmed by NMR spectroscopy. The proanthocyanidins in malt are structurally identical to those from barley.

Flavanol glycosides are well known compounds in nature where they appear as 3-, 5- and 7-*O*-glycosides and as 6- and 8-*C*-glucosides. The occurrence of these compounds is limited to a few sources [16, 17, 18]. In this paper we describe the structural elucidation of (2*R*,3*S*)-catechin-7-*O*-β-D-glucopyranoside isolated from barley malt and its quantitative change during malting. The compound was first isolated and described by

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Takani et al. in the wooden part of *Schizandra nigra* Max [19].

Materials and methods

Samples

For isolation, malt from the barley variety Prisma was used. Quantitative results were obtained from the investigation of 20 barley varieties (*Hordeum vulgare* L.) harvested in 1998 and from the corresponding malts. The malting conditions were as follows: 1 kg barley sample was steeped for two days at 14 °C to reach 45% steeping degree (immersing for 4 h in water, followed by 20 h air rest, repeated once), followed by five days germination at 14 °C. Each barley variety was cultivated at two different locations in Germany. The samples examined were: (i) spring barley: Alexis, Barke, Extract, Hanka, Krona, Luzon, Madeira, Madonna, Madras, Pasadena, Prosa, Ria, Ricarda, Santiago, Scarlett; (ii) winter barley: Angora, Clarine, Plaisant, Rifle, Tiffany.

Extraction and isolation procedures

Milling and extraction

A small amount of barley or malt was milled in a ball mill (MM 2000, Retsch, Haan, Germany) with liquid nitrogen-cooling for 3 minutes (amplitude 100%). About 4 g of the ground barley or malt was extracted with acetone/water (40 mL, 3:1, v/v) at room temperature for 1 hour in a rotating flask. Extracted meal was recovered by filtration through a Büchner funnel and extracted once again. The combined extracts were concentrated to approximately 5 mL in a rotary evaporator at 40 °C to remove the acetone.

Polyamide preparation

500 g polyamide CC 6 were shaken with deionised water (3.35 L) and methanol (625 mL) in a 5 L-plastic container. After overnight precipitation of the polyamide, the supernatant was removed, methanol (625 mL) was added and the procedure was repeated.

Polyamide column chromatography

A glass column (20×1.4 cm i.d.) was filled with cotton wool, sea sand, swollen polyamide (height 6 cm) and sea sand again. The polyamide was washed methanol-free with deionised water (100 mL). After the concentrated extract was applied to the top of the column, the flask was rinsed twice with deionised water (5 mL), and then the column washed with additional water (25 mL). The monomeric flavanols were eluted with methanol (30 mL). For semi-preparative HPLC the fractions of four columns were combined, concentrated to approximately 1.5 mL in a rotary evaporator, filtered through a 0.45 µm PTFE filter and applied to the semi-preparative HPLC system. For analytical investigations, each fraction was concentrated to about 1 mL, transferred to a 5 mL flask with acetic acid (2%)/acetonitrile (98:2, v/v), then filled up with the same solution, filtered through a 0.2 µm PTFE filter and injected into the analytical HPLC.

Semi-preparative HPLC

HPLC was conducted on a Beckman gradient system (high pressure) (Beckman Coulter, Unterschleißheim, Germany). Conditions were as follows: column, LiChroCART 250-10 LiChroSorb RP-18 (7 µm) (Merck, Darmstadt, Germany); mobile phase, 0.2% acetic acid (aq) and acetonitrile; gradient, 3.3% acetonitrile to 14.5% over 25 min to 75% acetonitrile over 5 min; flow, 4 mL min^{-1}; UV detection, 280 nm; DAD-UV spectra, 210–390 nm. Collected fractions were freeze-dried.

Analytical HPLC

HPLC analysis were performed on a ESA system (Chelmsford, MA, USA), consisting of two HPLC pumps, a high pressure gradient mixer, an additional pulse damper behind the mixer and an autosampler (cooled to 10 °C). The detection system was an online coupling of UV detection (Beckman Coulter, Unterschleißheim, Germany) and coulometric electrode array detection. The Coularray model 5600 (ESA) consisted of eight coulometric array cells with graphite working electrodes, palladium reference and platinum counter electrodes. The columns and the detector array were housed in a column thermostat at 30 °C. Conditions were as follows: column, LiChroCART 250-4 Superspher 100 RP-18 (4 µm) column (Merck, Darmstadt, Germany) with a LiChroCART 4-4 RP-18 (5 µm) guard column (Merck); mobile phase, A: 0.02 M NaH_2PO_4 adjusted to pH 3.4 with phosphoric acid and filtered through a 0.2 µm filter, B: 100 mL 0.1 M NaH_2PO_4 adjusted to pH 3.0 with phosphoric acid, 100 mL deionised water and 400 mL acetonitrile were mixed and filtered through a 0.2 µm PTFE filter; gradient, linear from 5% to 32% B in 40 min, to 90% B in 5 min; flow, 0.8 mL min^{-1}; UV detection, 280 nm; electrode array detection for eight electrodes, 0–770 mV increment 110 mV; internal standard, protocatechuic acid.

Identification procedures

Nuclear magnetic resonance (NMR)

1D- and 2D-NMR spectra were recorded at 300 K on a Bruker DMX 600 NMR spectrometer and on a Bruker ARX 400 NMR spectrometer (Bruker, Karlsruhe, Germany) using methanol-d_4 as solvent. Parameters were as follows: ^{1}H NMR (600.13 MHz); ^{13}C NMR (100.63 MHz); ^{1}H,^{1}H-COSY (400.13 MHz); 2D-multiple bond (HMBC, heteronuclear multiple bond correlation) ^{1}H-^{13}C-correlation: ^{1}H (600.13 MHz), ^{13}C (150.92 MHz).

Fast atom bombardment mass spectrometry (FAB-MS)

The spectrum of the isolated compound was recorded on a Conzept 1H mass spectrometer (Kratos, Manchester, UK) in the positive ion mode. 3-nitrobenzyl alcohol was used as matrix and ions were produced by bombardment with Xe atoms.

Circular dichroism (CD)

CD spectra were recorded in methanol using a Jasco J-720 spectropolarimeter (200–320 nm) (Jasco, Groß-Umstadt, Germany).

HPLC electrospray ionisation mass spectrometry (HPLC-ESI-MS)

Investigations were performed according to the method described previously [14].

Acid hydrolysis and sugar analysis

The isolated compound was hydrolysed under acidic conditions in the following manner: HCl (5 mL, c=3 mol L^{-1} in 50% ethanol) was added to the substance (ca. 0.2 mg) and the mixture was heated at reflux for 90 minutes. After evaporating to dryness under reduced pressure, acetic acid (2%)/acetonitrile (500 µL, 98:2, v/v) was added to the residue and the mixture was filtered through a 0.45 µm PTFE filter. This solution was benzoylated according to

the method described by Galensa [20, 21] with the following changes: volumes of reagents, pyridine (1 mL), benzoylchloride (125 µL), methanol (125 µL), water (20 mL); elution of the benzoates from the cartridges, isooctane/diethyl ether/acetonitrile (150:80:20, v/v/v) in a 20 mL flask; reference substances D-(–)-fructose, D-(+)-galactose, D-(+)-glucose, D-(+)-mannose.

HPLC analysis of the benzoates

HPLC analysis of the benzoates was performed on an isocratic system (Beckman Coulter, Unterschleißheim, Germany). Conditions were as follows: column, Hypersil Si, 3 µm (250×4.6 mm i.d.) (CS Chromatographie Service, Langerwehe, Germany); mobile phase, isooctane/diethyl ether/acetonitrile (150:55:2, v/v/v); flow, 0.8 mL min^{-1}; UV detection, 230 nm.

Enzyme hydrolysis

The isolated compound was hydrolysed under enzymatic conditions in the following manner: technical enzyme suspension (2 mL Pectinol) (Röhm, Darmstadt, Germany) and water (500 µL) were added to the isolated compound (ca. 0.2 mg). The mixture was incubated 24 h at 37 °C and then filtered through a 0.2 µm PTFE filter. The solution was applied to the analytical HPLC system described above.

Results and discussion

This paper deals with the determination of monomeric flavanols in barley and malt samples. Figure 1 shows reversed phase HPLC chromatograms of an acetone/water extract from malt after polyamide clean-up. Compound 2 was identified as (+)-catechin by co-chromatography and on the basis of its hydrodynamic voltammogram and UV spectrum compared to the reference compound. Additionally, a molecular ion at *m/z* 289=$[M–H]^-$ was detected with HPLC electrospray ionisation mass spectrometry (HPLC-ESI-MS) analysis. In contrast, (–)-epicatecin was not detected.

ESI-MS analysis of compound 1 using a method described previously [14], revealed a molecular ion at *m/z* 451=$[M–H]^-$ in the negative ion mode and a fragment ion at *m/z* 289. Therefore, a catechin hexoside was suspected. Only two publications supported this postulate: (i) The same masses were detected by Roeder et al. with HPLC thermospray-MS who also assumed a catechin glycoside in malt [22]; (ii) A catechin glycoside was detected with gas chromatography mass spectrometry analysis in beer after trimethylsilylation, but no further details regarding the nature and linkage of the sugar unit were given [23].

Identification of compound 1

Compound 1 was isolated by semi-preparative HPLC and identified as (2*R*,3*S*)-catechin-7-*O*-β-D-glucopyranoside (Fig. 2) on the basis of spectroscopic and chromatographic data. The data were as follows: λ_{max} 225 and 277 nm; ^{1}H NMR (methanol-d_4, $\delta_{methanol}$=3.35 ppm) δ [ppm]: 6.87 (1 H, d, *J*=1.9 Hz, H-2′), 6.80 (1 H, d, *J*=8.1 Hz, H-5′), 6.75 (1 H, dd, *J*=8.1, 1.9 Hz, H-6′), 6.24 (1 H, d, *J*=2.2 Hz, H-6), 6.20 (1H, d, *J*=2.4 Hz, H-8), 4.86 (n.d., d, *J*=7.6 Hz, H-1″), 4.63 (1 H, d, *J*=7.4 Hz, H-2), 4.04 (1 H, m, H-3), 3.92 (1 H, dd, *J*=11.9, 1.6 Hz, H-6″ B), 3.73 (1 H, dd, *J*=12.0, 4.9 Hz, H-6″ A), 3.50–3.40 (4 H, H-3″, H-4″, H-5″ (3.44 ppm), H-2″ (3.43 ppm)), 2.90 (1 H, dd, *J*=16.3, 5.4 Hz, H-4 A), 2.58 (1 H, dd, *J*=16.3, 8.0 Hz, H-4 B); ^{13}C NMR (Table 1).

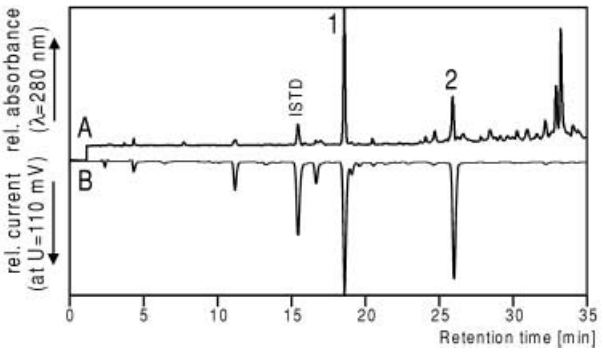

Fig. 1 Chromatograms of analytical reversed phase HPLC recorded at **A** 280 nm and **B** 110 mV. *ISTD* internal standard, *1* (2*R*,3*S*)-catechin-7-*O*-β-D-glucopyranoside, *2* (+)-catechin

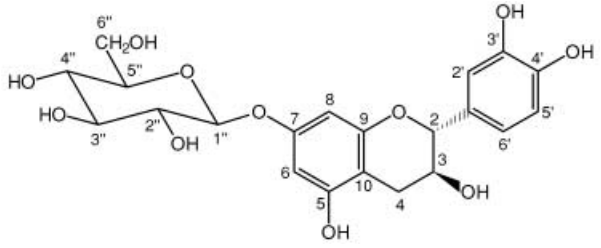

Fig. 2 Structure of (2*R*,3*S*)-catechin-7-*O*-β-D-glucopyranoside

The ^{1}H and ^{13}C NMR spectra suggested that the compound had a catechin structure and a sugar moiety. The signals of the aliphatic protons are characteristic of catechins. The coupling constant of H-2 (*J*=7.4 Hz) confirm a 2,3-*trans* configuration of the protons [24, 25]. The signals at 6.24 and 6.20 ppm were assigned to H-6 and H-8 from the ^{1}H,^{13}C-HMBC spectrum. The data of the catechin aglycon comply with the data in the literature [24, 26, 27]. Compared to catechin, the signals of H-6 and H-8 were shifted downfield. Moreover, the HMBC spectrum confirmed that the sugar moiety was linked to C-7 of the skeleton. The carbon resonances were assigned on the basis of increment systems, comparison with data in the literature [26, 28, 29, 30] and the HMBC spectrum. The signal for the anomeric proton of the sugar moiety appears at 4.86 ppm with a coupling constant of 7.6 Hz to H-2″. This indicates a transdiaxial configuration of H-1″ and H-2″ and the sugar was determined to be a β-pyranose [24, 31]. The identity of the sugar was determined to be β-glucopyranoside on the basis of the relative shift of the six aliphatic carbon signals of the sugar moiety and the coupling constants of

Table 1 ^{13}C NMR spectral data of (2*R*,3*S*)-catechin-7-*O*-β-D-glucopyranoside (100 MHz) and comparison with data taken from literature. Chemical shift δ/ppm (methanol-d_4, δ=49.0 ppm)

Carbon	(2*R*,3*S*)-Catechin-7-*O*-β-D-glucopyranoside	(+)-Catechin-7-*O*-β-D-glucopyranoside in acetone-d_6/D_2O [26]	(+)-Catechin [29]	β-Glucose [30]
2	83.0	81.6	82.0	
3	68.6	67.2	67.9	
4	28.6	27.5	28.1	
5	157.6	156.7	157	
6	97.5	97.5	97	
7	158.7	156.5	157	
8	97.0	96.3	96	
9	156.9	155.5	156	
10	103.7	102.8	102	
1′	132.1	130.9	132	
2′	115.3	115.1	115	
3′	146.3	145.0	145	
4′	146.3	144.8	145	
5′	116.1	116.0	116	
6′	120.0	119.7	120	
1″	102.3[a]	101.1[a]		96.7
2″	74.9	73.4		75.1
3″	78.1	76.4		76.7
4″	71.4	70.1		70.6
5″	78.1	76.7		76.8
6″	62.6	61.3		61.7

[a] shifted downfield due to glycosidic linkage

H-1″ and H-6″ to their respective protons [26, 30, 32]. Additionally, molecular ions at *m/z* 453=$[M+H]^+$ and *m/z* 475=$[M+Na]^+$ were detected by FAB-MS analysis.

Further investigations were performed to confirm the identity of the aglycon and the sugar moiety. Enzymatic hydrolysis yielded a complete degradation of compound 1 and the formation of a new substance that was identified as (+)-catechin on the basis of co-chromatography, HPLC-ESI-MS results and its hydrodynamic voltammogram compared to the reference standard. After acid hydrolysis of compound 1 followed by derivatisation (benzoylation), the hydrolysis product was confirmed to be glucose by co-chromatography with benzoylated reference standards.

The absolute stereochemistry at C-2 was determined by CD spectroscopy. For flavan-3-ols, this method is mainly used to evaluate the configuration at C-4 in oligomeric proanthocyanidins due to the Cotton effect at 200–230 nm [18, 33]. Flavanols with a (2*R*)-configuration show a negative Cotton effect at 280 nm that is independent of the configuration at C-3. Therefore, the negative Cotton effect at 281 nm in the CD spectrum of (+)-catechin (Fig. 3) was in compliance with the literature [34]. The CD spectrum of compound 1 showed a negative Cotton effect at 279 nm. In combination with the 2,3-*trans* configuration determined by NMR, it was clearly demonstrated that the substance has a (2*R*,3*S*)-configuration.

From these data, compound 1 was identified as (2*R*,3*S*)-catechin-7-*O*-β-D-glucopyranoside. This flavanol glycoside is described in barley (*Hordeum vulgare* L.) and malt for the first time.

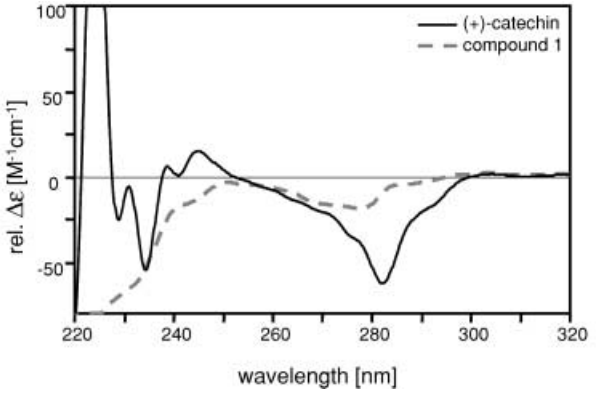

Fig. 3 CD spectra of (2*R*,3*S*)-catechin-7-*O*-β-D-glucopyranoside (compound 1) and (+)-catechin reference standard in methanol

Content of monomeric flavanols in barley and malt

The amount of (+)-catechin and (2*R*,3*S*)-catechin-7-*O*-β-D-glucopyranoside in samples of different barley varieties and the corresponding malts was determined by HPLC. Quantification was performed by calibration with a (+)-catechin reference standard. Data obtained are presented in Table 2.

The amount of (+)-catechin in the investigated barley varieties covered the range 23–70 mg kg^{-1} dry weight (average: 39 mg kg^{-1}); the catechin glucoside covered the range 6–38 mg kg^{-1} dry weight (average: 18 mg kg^{-1}). During malting the content of catechin significantly decreased to 14–32% of the initial value. This effect was in agreement with other results in the literature from which it can be concluded that the amount of proanthocyanidins and catechins remained nearly unchanged during malting or decreases [9, 35, 36, 37]. Nevertheless, changes of polyphenols during malting are

Table 2 Amount of (2*R*,3*S*)-catechin-7-*O*-β-D-glucopyranoside and (+)-catechin (mg kg^{-1} dry weight) in barley and corresponding malt determined by HPLC with UV detection (λ=280 nm)

Variety	(2*R*,3*S*)-Catechin-7-*O*-β-D-glucopyranoside		(+)-Catechin	
	barley	malt	barley	malt
location 1				
Alexis	8.0	37.8	45.2	13.0
Scarlett	15.4	31.8	33.0	9.5
Madras	6.6	27.1	34.5	10.6
Madonna	22.0	33.1	32.7	7.3
Hanka	12.4	31.8	35.1	6.7
Prosa	19.0	42.7	41.2	12.8
Pasadena	29.6	36.6	22.7	6.8
Ria	18.8	50.0	49.8	11.1
Madeira	16.6	33.7	33.7	9.7
Santiago	11.5	33.1	37.5	9.2
Ricarda	7.9	38.1	48.8	9.6
Luzon	8.2	32.5	42.9	12.6
Extract	6.2	26.9	29.3	8.1
Barke	6.4	28.9	35.1	10.7
Krona	17.9	27.3	26.1	6.1
location 2				
Alexis	10.8	42.8	51.7	12.0
Scarlett	24.8	38.4	32.5	6.1
Madras	9.2	33.4	32.5	9.2
Madonna	33.3	32.8	26.7	7.0
Hanka	18.9	29.9	32.9	7.0
Prosa	17.6	42.6	50.9	10.9
Pasadena	37.9	37.0	24.0	5.9
Ria	19.8	53.1	54.4	8.7
Madeira	19.4	33.1	32.5	7.8
Santiago	15.5	37.3	38.4	8.5
Ricarda	16.0	45.3	43.6	8.5
Luzon	10.1	37.3	40.5	9.2
Extract	14.8	32.3	35.2	6.1
Barke	7.6	34.8	37.2	7.4
Krona	17.3	25.0	30.4	7.4
Plaisant	30.6	44.8	53.7	28.3
Angora	33.1	50.1	50.9	13.6
Clarine	25.2	35.5	36.8	15.4
Rifle	32.4	67.5	70.4	23.9
Tiffany	21.4	39.5	42.4	13.0
location 3				
Plaisant	17.1	33.2	39.1	15.0
Angora	17.7	29.5	45.9	6.6
Clarine	13.5	26.1	30.8	10.0
Rifle	22.6	49.5	43.7	12.4
Tiffany	15.1	40.3	48.3	9.9

not finally elucidated. The obtained amounts of catechin are in compliance with data in the literature [9, 35].

In contrast, the amount of catechin glucoside significantly increased during malting. The reasons could be as follows: (i) biosynthesis of the compound during development of the sprout; (ii) an existing compound in barley could be made available for extraction by any occurrences during malting. According to a theory of Beart et al. [38], the proanthocyanidins from plants are partially covalently bound to a polysaccharide matrix. The glucoside could be released from this during malting directly, by activated or developed enzymes.

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