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Identification of Two Novel Prodelphinidin A-Type Dimers from Roasted Hazelnut Skins (*Corylus avellana* L.)

Tuba Esatbeyoglu,[†] Victor Wray,[§] and Peter Winterhalter*[,†]

[†]Institute of Food Chemistry, Technische Universität Braunschweig, Schleinitzstrasse 20, 38106 Braunschweig, Germany

[§]Helmholtz Centre for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany

ABSTRACT: Two new A-type dimeric prodelphinidins, EGC-(2β→O7, 4β→8)-C and EGC-(2β→O5, 4β→6)-C, were isolated from the skins of roasted hazelnut (*Corylus avellana* L.) by low-speed rotary countercurrent chromatography (LSRCCC) and final purification by preparative HPLC. Their structures were determined by a combination of mass spectrometry (HPLC-ESI-MS^n and HR-ESI-MS) and NMR spectroscopy that included the application of 2D methods (^{1}H−^{1}H COSY, HSQC, HMBC, and NOESY). Furthermore, circular dichroism (CD) and acid-catalyzed degradation (phloroglucinolysis) confirmed the proposed structures.

KEYWORDS: *hazelnut, A-type proanthocyanidins, ^{1}H and ^{13}C NMR, circular dichroism, phloroglucinolysis*

INTRODUCTION

Proanthocyanidins are of great interest in medicine because of their possible protective effects on human health in reducing the risk of chronic diseases.[1] Proanthocyanidins are some of the most ubiquitous phenolic compounds in our diet. Proanthocyanidins, also known as condensed tannins, containing (epi)catechin, (epi)afzelechin, or (epi)gallocatechin as flavan-3-ol units, are named procyanidins, propelargonidins, or prodelphinidins, respectively. In the so-called B-type proanthocyanidins the flavan-3-ol units are linked through C4→C8 or C4→C6, respectively. A-Type proanthocyanidins have a second linkage through an ether bond at C2→O7.[2,3] A-Type proanthocyanidins are present in only a limited number of foods such as peanuts, plums, cranberries, avocados, and cinnamon.[2]

Hazelnut (*Corylus avellana* L.), which belongs to the Betulaceae family, is a well-known tree nut mainly distributed along the coasts of the Black Sea region of Turkey.[4,5] Turkey is the world's largest hazelnut producer followed by Italy, accounting for about 70% of worldwide production with a crop of 430,000 tons in 2011.[6] Hazelnut skins, which contain the majority of phenolics including condensed tannins,[5,7−9] are byproducts of the roasting process, and the pharmaceutical and food industries have an interest in using these side streams as natural antioxidants and possible functional food ingredients.[5,10] Many health benefits of hazelnut, such as antioxidant activity that in the long-term may lower the risk for cardiovascular diseases, have been investigated.[5,11]

Pelvan et al. showed that roasting has a significant effect on the loss of total phenolics and condensed tannins due to the removal of the skin.[8] Therefore, hazelnuts should be consumed with the skins.[8,9] Moreover, roasted hazelnuts contained comparable amounts of phenolics as unroasted hazelnuts without skins.[9] Roasted skins from hazelnuts are mostly composed of B-type procyanidins or procyanidin−prodelphinidin heteropolymers up to a degree of polymerization of 7,[10] whereas the occurrence of A-type proanthocyanidins in either hazelnuts or their skins has not been reported.[2,10,12] In the present study, isolation and structural characterization of two new A-type prodelphinidins that occur naturally in hazelnut skins will be presented.

MATERIALS AND METHODS

Chemicals. Acetonitrile of HPLC quality (Fisher Scientific, Loughborough, UK), *tert*-butyl methyl ether (distilled, industrial quality), *n*-butanol, p.a. (Fisher Scientific), water (deionized, Nanopure, Werner, Leverkusen, Germany), *n*-hexane (distilled, industrial quality), ethanol (distilled, industrial quality), acetone (distilled, industrial quality), methanol (distilled, industrial quality), hydrochloric acid, 37% (Riedel-de-Haën, Seelze, Germany), acetone-d_6, and methanol-d_4 (Deutero GmbH, Kastellaun, Germany), and methanol for spectroscopy (Uvasol, Merck, Darmstadt, Germany) were used.

Roasted hazelnut skins were kindly supplied by Wild Flavors GmbH & Co. KG (Berlin, Germany).

High-Performance Liquid Chromatography Photodiode Array (HPLC-PDA). HPLC-PDA conditions were the same as described earlier.[13]

Sample Preparation for Low-Speed Rotary Countercurrent Chromatography (LSRCCC) Separation. Hazelnut skins were defatted three times with *n*-hexane and extracted three times with 70% aqueous acetone (v/v). The acetone extracts were evaporated and freeze-dried. The freeze-dried 70% aqueous acetone extract of hazelnut skins was stirred for 1 h in ethanol, and the insoluble residues were filtered. *n*-Hexane was added at a flow rate of 10 mL/min to the solution (ethanol/*n*-hexane (5:13, v/v). The precipitate was filtered, dissolved in water, and lyophilized.

To form dimeric procyanidins B1, B3, B6, and B7, 10 g of (+)-catechin and 10 g of hazelnut skin precipitate were dissolved in 500 mL of 0.1 N methanolic HCl and stirred at 40 °C for 30 min in a water bath. The reaction was stopped with 0.5 N sodium hydrogen carbonate (in water). The solution was then evaporated under vacuum and lyophilized.

The reaction mixture obtained was stirred for 1 h in ethanol, the insoluble ethanol residue was filtered, and *n*-hexane was added

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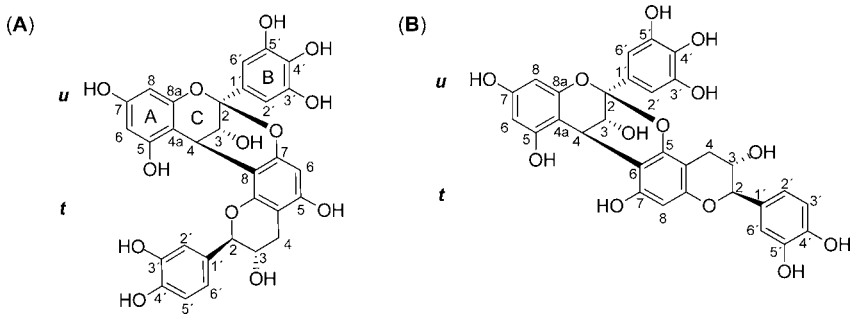

Figure 1. Chemical structures of EGC-(2*β*→O7, 4*β*→8)-C (A) and EGC-(2*β*→O5, 4*β*→6)-C (B).

dropwise (10 mL/min; ethanol/*n*-hexane (5:13, v/v). After filtration of the precipitate, the filtrate was evaporated and the residue was dissolved in water and lyophilized.

LSRCCC Separation. A preparative prototype low-speed countercurrent chromatograph (Pharma-Tech Research Corp., Baltimore, MD, USA) was equipped with a multilayer single-coil column (diameter of chemically inert PTFE tubing = 8.2 mm, total capacity = 5500 L, revolution speed = 50 rpm). For the separation by LSRCCC, 9.58 g of the lyophilized filtrate of the semisynthetic process (unpublished data) dissolved in 200 mL of a 1:1 (v/v) mixture of upper and lower phase was injected via a sample loop. The two-phase solvent system was composed of *tert*-butyl methyl ether/*n*-butanol/water (4.3:0.7:5, v/v/v). The upper organic phase represented the mobile phase. The LSRCCC separation was performed at a flow rate of 5.0 mL/min (HPLC Pump 64, Knauer, Berlin, Germany) and was carried out in the "head-to-tail" elution mode. Elution was monitored with a Knauer UV−vis detector (Berlin, Germany) at λ = 280 nm and recorded by a Servogor 120 plotter (BBC Goerz Metrawatt SE 120, Vienna, Austria). Fractions were collected with a fraction collector (Pharmacia LKB Super Frac, Bromma, Sweden). After a separation duration of 29 h, the elution mode was switched to extrusion by pumping the aqueous lower phase into the column.

Preparative HPLC. An HPLC system from Knauer (Berlin, Germany) consisting of a Smartline 1000 HPLC pump, a Smartline Manager 5000 solvent organizer and degasser, a Wellchrom K-2600 UV detector, a Rheodyne 7125 injector (200 μL), and ChromGate V3.1.7 software was used. The preparative HPLC column (Hypersil ODS C-18 5 μm, 250 × 16 mm i.d., Phenomenex, Aschaffenburg, Germany) was operated with a binary solvent system of water (solvent A) and acetonitrile (solvent B) at a flow rate of 6 mL/min. The following gradient was used for the isolation of EGC-(2*β*→O7, 4*β*→8)-C: 0 min, 6% B; 50 min, 27% B. For the isolation of EGC-(2*β*→O5, 4*β*→6)-C the gradient was as follows: 0 min, 10% B; 45 min, 25% B. The chromatograms were monitored at λ = 280 nm. The purity of both compounds was confirmed as >95% by HPLC-PDA at λ = 280 nm.

HPLC−Electrospray Ionization Multiple Mass Spectrometry (HPLC-ESI-MS^n). HPLC-ESI-MS^n analysis was carried out according to ref 13.

High-Resolution ESI-MS (HR-ESI-MS). High-resolution ESI-MS spectra were recorded on a Thermo Science LTQ Orbitrap mass spectrometer in the positive ionization mode (Thermo Fisher Scientific, Bremen, Germany).

Phloroglucinolysis. Samples for phloroglucinolysis were prepared as described earlier.[14]

Circular Dichroism (CD). CD spectra were measured in methanol (<0.2 mmol/L in methanol) at room temperature using a Jasco J-715 CD spectropolarimeter (Gross-Umstadt, Germany), Spectra Manager version 1.55.00 software, and quartz cuvettes (Hellma SuprasilR 300, type: 100-QX, *d* = 1 mm). Scan parameters were as follows: wavelength, 200−400 nm; bandwidth, 1.0 nm; sensitivity, 100 mdeg; response, 1 s; scan speed, 50 nm/min; step resolution, 0.1 nm.

Nuclear Magnetic Resonance (NMR) Spectroscopy. ^{1}H, ^{13}C, ^{1}H−^{1}H COSY, ^{1}H−^{1}H phase-sensitive NOESY, HSQC, and HMBC experiments were carried out at 300 or 240 K on Bruker Avance DMX 600 or ARX 400 NMR spectrometers equipped with a variable-temperature unit B VT-2000 (Rheinstetten, Germany), respectively. Chemical shifts were referenced to the solvent signal acetone-d_6 or methanol-d_4. Chemical shifts are given in parts per million and coupling constants in hertz.

(−)-Epigallocatechin-(2*β*→O7, 4*β*→8)-(+)-catechin: amorphous white powder; λ_{max} = 228 and 276 nm; ESI-MS/MS *m/z* 591 $[M - H]^-$; MS/MS fragments *m/z* 573, 465, 453, 447, 439, 407, 301, 289, 245; HR-ESI-MS *m/z* 593.1293 $[M + H]^+$ (calcd for 593.1290 $C_{30}H_{24}O_{13}$); CD (0.18 mmol/L in methanol) $[\theta]_{203}$ 77458, $[\theta]_{211}$ −5086, $[\theta]_{224}$ 33614, $[\theta]_{238}$ 24522, $[\theta]_{271}$ −13695, $[\theta]_{285}$ 102.

^{1}H NMR (400 MHz, acetone-d_6, 240 K) *δ* 2.53 (dd, 1H, *J* = 9.4 Hz, *J* = 16.4 Hz, H4B*t*), 3.02 (dd, 1H, *J* = 5.9 Hz, *J* = 16.5 Hz, H4A*t*), 4.09 (d, 1H, *J* = 3.5 Hz, H3*u*), 4.13−4.16 (m, 1H, H4*u*), 4.16 (m, 1H, H3*t*), 4.61 (1OH, OH3*t*), 4.62 (d, 1H, *J* = 8.9 Hz, H2*t*), 4.70 (1OH, OH3*u*), 5.94 (d, 1H, *J* = 2.2 Hz, H6*u*), 6.02 (d, 1H, *J* = 2.2 Hz, H8*u*), 6.11 (s, 1H, H6*t*), 6.69 (s, 2H, H2′*u*, H6′*u*), 6.87 (dd, 1H, *J* = 1.8 Hz, *J* = 8.1 Hz, H6′*t*), 6.83 (d, 1H, *J* = 8.1 Hz, H5′*t*), 7.01 (d, 1H, *J* = 1.8 Hz, H2′*t*).

^{13}C NMR (100 MHz, acetone-d_6, 240 K) *δ* 29.0 (C4*t*), 29.4 (C4*u*), 66.8 (C3*u*), 66.9 (C3*t*), 84.1 (C2*t*), 95.6[a] (C6*t*), 95.9[a] (C8*u*), 97.3 (C6*u*), 99.5 (C2*u*), 102.6 (C4a*t*), 103.3 (C4a*u*), 106.2 (C8*t*), 106.9 (C2′*u*, C6′*u*), 115.5 (C2′*t*), 115.5 (C5′*t*), 120.4 (C6′*t*), 129.5 (C1′*t*), 130.6 (C1′*u*), 133.1 (C4′*u*), 145.2 (C3′*u*), 145.2 (C5′*u*), 145.6 (C3′*t*), 145.6 (C4′*t*), 150.5 (C8a*t*), 151.3 (C7*t*), 153.5 (C8a*u*), 155.1 (C5*t*), 156.0 (C5*u*), 157.4 (C7*u*) ([a], shifts are interchangeable).

^{1}H NMR (600 MHz, methanol-d_4, 300 K) *δ* 2.61 (dd, 1H, *J* = 8.2 Hz, *J* = 16.4 Hz, H4B*t*), 2.98 (dd, 1H, *J* = 5.6 Hz, *J* = 16.4 Hz, H4A*t*), 4.10 (d, 1H, *J* = 3.5 Hz, H3*u*), 4.19 (m, 1H, H3*t*), 4.26 (d, 1H, *J* = 3.5 Hz, H4*u*), 4.77 (d, 1H, *J* = 7.8 Hz, H2*t*), 5.99 (d, 1H, *J* = 2.3 Hz, H6*u*), 6.11 (d, 1H, *J* = 2.3 Hz, H8*u*), 6.13 (s, 1H, H6*t*), 6.76 (s, 2H, H2′*u*, H6′*u*), 6.85 (m, 2H, H5′*t*, H6′*t*), 6.95 (d, 1H, *J* = not determined, H2′*t*).

^{13}C NMR (150 MHz, methanol-d_4, 300 K) *δ* 27.6 (C4*t*), 28.1 (C4*u*), 66.4 (C3*u*), 66.8 (C3*t*), 83.3 (C2*t*), 95.0 (C8*u*), ∼96 (C6*t*), 96.8 (C6*u*), 98.9 (C2*u*), 101.7 (C4a*t*), 102.7 (C4a*u*), 105.5 (C8*t*), ∼107 (C2′*u*, C6′*u*), 114.5 (C2′*t*), 114.5 (C5′*t*), 119.4 (C6′*t*), 129.2 (C1′*t*), 130.3 (C1′*u*), 133.2 (C4′*u*), 145.2 (C3′*u*), 145.2 (C5′*u*), 145.3 (C3′*t*), 145.3 (C4′*t*), 150.1 (C8a*t*), 151.0 (C7*t*), 152.9 (C8a*u*), 154.9 (C5*t*), 155.6 (C5*u*), 156.7 (C7*u*).

(−)-Epigallocatechin-(2*β*→O5, 4*β*→6)-(+)-catechin: amorphous white powder; λ_{max} = 228 and 276 nm; ESI-MS/MS *m/z* 591 $[M - H]^-$; MS/MS fragments *m/z* 573, 465, 453, 447, 439, 407, 301, 289, 245; HR-ESI-MS *m/z* 593.1289 $[M + H]^+$ (calcd for 593.1290 $C_{30}H_{24}O_{13}$); CD (0.17 mmol/L in methanol) $[\theta]_{204}$ −269050, $[\theta]_{215}$ 107968, $[\theta]_{240}$ 18830, $[\theta]_{271}$ 13355, $[\theta]_{288}$ 3757.

^{1}H NMR (600 MHz, acetone-d_6, 300 K) *δ* 2.62 (dd, 1H, *J* = 8.2 Hz, *J* = 16.3 Hz, H4B*t*), 2.85 (dd, 1H, *J* = 5.5 Hz, *J* = 16.3 Hz, H4A*t*), 3.98 (m, 1H, H3*t*), 4.15 (d, 1H, *J* = 3.3 Hz, H3*u*), 4.33 (d, 1H, *J* = 3.5 Hz, H4*u*), 4.60 (d, 1H, *J* = 7.6 Hz, H2*t*), 6.05 (s, 1H, H8*t*), 6.08 (d, 1H, *J* = 2.3 Hz, H6*u*), 6.09 (d, 1H, *J* = 2.3 Hz, H8*u*), 6.71 (dd, 1H, *J* = 2.0 Hz, *J* = 8.1 Hz, H6′*t*), 6.76 (d, 1H, *J* = 8.1 Hz, H5′*t*), 6.83 (s, 2H, H2′*u*, H6′*u*), 6.84 (d, 1H, *J* = 2.0 Hz, H2′*t*).

^{13}C NMR (150 MHz, acetone-d_6, 300 K) *δ* 29.4 (C4*t*), 29.5 (C4*u*), 67.3 (C3*u*), 67.8 (C3*t*), 82.7 (C2*t*), 96.4 (C8*u*), 97.2 (C8*t*), 97.4

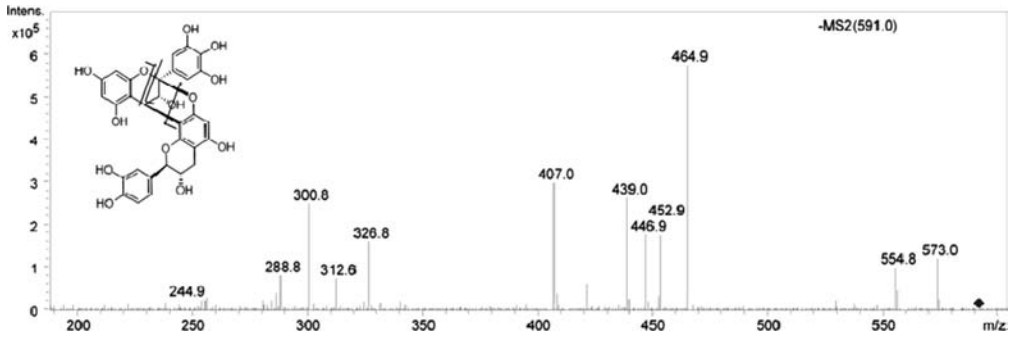

Figure 2. MS/MS fragmentation pattern of EGC-(2β→O7, 4β→8)-C ([M − H]$^-$ m/z 591) and EGC-(2β→O5, 4β→6)-C ([M − H]$^-$ m/z 591), consistent with a structure composed of (epi)gallocatechin in the upper unit and (epi)catechin in the terminal unit.[46]

(C6u), 100.3 (C2u), 101.9 (C4at), 104.2 (C4au), 107.6 (C2′u, C6′u), 108.0 (C6t), 115.0 (C2′t), 115.7 (C5′t), 119.9 (C6′t), 131.3 (C1′u), 131.9 (C1′t), 133.9 (C4′u), 145.6[a] (C3′u), 145.6[a] (C5′u), 145.7[a] (C3′t), 145.7[a] (C4′t), 151.0 (C8at), 152.1 (C7t), 154.2 (C8au), 155.0 (C5t), 155.6 (C5u), 157.9 (C7u) ([a], shifts are interchangeable).

RESULTS AND DISCUSSION

Two new prodelphinidin A-type dimers EGC-(2β→O7, 4β→8)-C and EGC-(2β→O5, 4β→6)-C in amounts of 5−10 mg were obtained from a polymeric hazelnut (*C. avellana* L.) skin fraction after semisynthesis and enrichment by LSRCCC followed by a final purification by preparative HPLC. EGC-(2β→O7, 4β→8)-C was isolated as a white amorphous powder from the coil fraction, and EGC-(2β→O5, 4β→6)-C was obtained as a white amorphous powder from fraction I of the LSRCCC after separation by preparative HPLC. The chemical structures of both compounds are shown in Figure 1. The structures of these new compounds were determined on the basis of low- and high-resolution electrospray ionization mass spectrometry as well as analysis by ^{1}H NMR, ^{13}C NMR, and 2D-NMR spectroscopy that included COSY, HSQC, HMBC, and NOESY.

Structure Elucidation of Two New A-Type Prodelphinidins by ESI-MS. LC-MS/MS analysis yielded in both cases a molecular ion of [M − H]$^-$ m/z 591, which indicated the presence of an A-type prodelphinidin. Figure 2 shows the similar fragmentation pattern of [M − H]$^-$ m/z 591 of both compounds with the fragment ion m/z 465 resulting from a characteristic heterocyclic ring fission (C-ring). The quinone methide fission produced two dimers: m/z 301 and 289, representing an (epi)gallocatechin and (epi)catechin unit (upper and terminal unit). The retro-Diels−Alder fission of m/z 439 indicated a loss of m/z 152, indicating the terminal unit is an (epi)catechin.[15] On the basis of these results, we concluded that (epi)gallocatechin was the upper unit and (epi)catechin the terminal unit.[16,17] The absence of m/z 305 confirmed this assumption.[16] Thus, the position of the flavan-3-ol units in both compounds was clear, but it was not possible to distinguish between the possible linkages of the units involved.

Compounds **A** EGC-(2β→O7, 4β→8)-C and **B** EGC-(2β→O5, 4β→6)-C showed, besides the LC-MS/MS analysis, high-resolution ESI-MS with quasi-molecular ions at m/z 593.1293 and 593.1289 [M + H]$^+$, respectively, indicating identical molecular formulas of $C_{30}H_{24}O_{13}$ (calcd m/z 593.1290).

Structure Elucidation of EGC-(2β→O7, 4β→8)-C (Compound A). B-Type proanthocyanidins are connected through a single C4u−C8t or C4u−C6t linkage, whereas A-type proanthocyanidins have an additional C−O−C linkage (ether linkage) between C2u and O−C7t or O−C5t. As a consequence, A-type proanthocyanidins are rigid molecules, which allow NMR spectra to be recorded at room temperature. All ^{1}H and ^{13}C NMR resonances were assigned by 1D and 2D ^{1}H and ^{13}C NMR spectroscopic analysis that included HSQC, HMBC, COESY, and NOESY at 300 or 240 K (400 MHz, acetone-d_6). The ^{1}H and ^{13}C chemical shifts and coupling constants are summarized under Materials and Methods.

The position of meta-substitution A-ring (phloroglucinol ring) protons H6u (δ 5.94) and H8u (δ 6.02) were assigned from NOE correlations between H8u and H2′u as well as H6′u.

^{13}C NMR spectra of A-type are very similar to those of B-type proanthocyanidins. C2′ and C6′ signals for trihydroxylated B-ring C-atoms (pyrogallol ring) appear at δ ∼108 and C2′, C5′, and C6′ signals for dihydroxylated B-ring C-atoms (catechol ring) at δ 115−120.[18] Furthermore, an aromatic two-proton singlet at δ 6.69 or 6.76 is typical for H2′ and H6′, indicating a trisubstituted B-ring.[18] Significant differences are found in the chemical shifts of the C-ring carbons C2 and C4 of the upper unit due to the oxygen substituent at C2; thus, C2 is downfield of C4.[19] ^{1}H NMR spectra showed a characteristic AB coupling system for C-ring protons of the upper unit (H3u δ 4.09, doublet ($J_{3,4}$ = 3.5 Hz); H4u δ 4.13−4.16, multiplet through coupling to H3u and the 3u−OH in acetone-d_6), which is typical for A-type proanthocyanidins. Also, the coupling constant of $J_{3,4}$ = 3.5 Hz for the C-ring protons and the chemical shift of δ 99.5 for a ketal carbon at the C2u-position of the upper unit is representative for A-type proanthocyanidins.[20−22]

The relative position of the two flavan-3-ol units was confirmed from COSY and HMBC data. The 3′,4′-substitution of the B-ring (catechol ring) of the terminal unit was indicated by the long-range correlation of H2t (δ 4.62) with C1′t (δ 129.5), C2′t (δ 115.5), and C6′t (δ 120.4), whereas H2′u and H6′u correlations (δ 6.69, singlet) with C2u (δ 99.5) verified the 3′,4′,5′-substitution of the B-ring (pyrogallol ring) of the upper unit.

The doublet for H2t (δ 4.62) with the coupling constant of 8.9 Hz indicated a 2,3-*trans* configuration. Consequently, the terminal unit is composed of (+)-catechin and was confirmed by the ^{13}C signal of C2t at δ 84.1.[19,23−27] The upper unit was apparent from the chemical shifts of H3u (δ 4.09) and C3u (δ 66.8) according to Baldé et al.[27−29] and suggested (−)-epigallocatechin was present as the upper unit.[30] The configuration of the upper unit and the linkage between the two monomers are clarified below.

The ^{13}C NMR chemical shift of C2u (δ 99.5) implied that the C−O−C linkage between units was at C2 and, hence, the

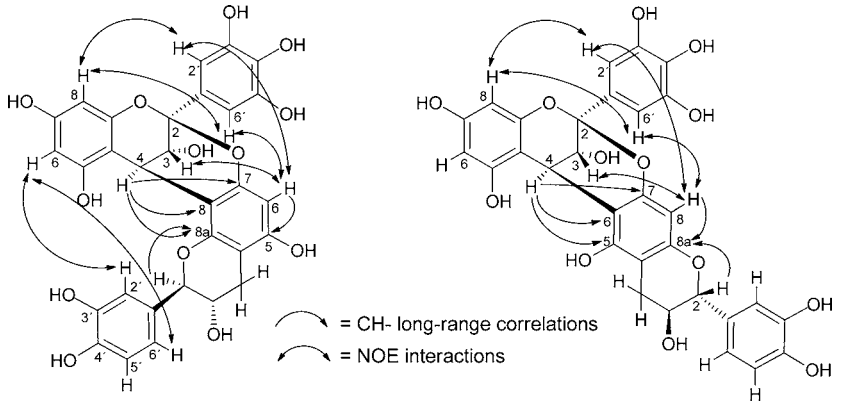

Figure 3. Selected CH long-range correlations and NOE interactions for determination of the nature of the interflavanoid linkage (4→8 and 4→6).[46]

C−C linkage was at C4*u*. Thus, in principle, only three A-type proanthocyanidins can be envisaged: 2*β*→O7, 4*β*→8; 2*β*→O7, 4*β*→6; and 2*β*→O5, 4*β*→6, as the C8-ring resulting from the 2*β*→O5, 4*β*→8 connection is structurally impossible.[31] The assignment of the interflavanoid linkage was determined from NOE interactions in the NOESY spectrum and HMBC correlations. H2′*u* and H6′*u* interacted with H8*u* and H6*t* or H8*t*; the latter correlation is a unique confirmation of the 2*uβ*→O7*t* bond. On the basis of these results the compound has either 2*uβ*→O7*t*, 4*uβ*→8*t* or 2*uβ*→O7*t*, 4*uβ*→6*t* linkages. The proton distance between H2′*u*/H6′*u* and H6*t* as well as H8t is identical for both linkage types (4*uβ*→8*t* and 4*uβ*→6*t*). A possible method for the determination of the C−C linkage for A-types is through comparison of chemical shifts of H2*t* with those of B-types.[26,32,33] This, however, was not unambiguous. According to other authors it is possible to determine the 4→8 interflavanoid bond through correlations between H4*u* and H2′*t*/H6′*t* (B-ring).[34,35] In acetone-d_6 the H4*u* and H3*t* protons, which correlate with the B-ring protons of the terminal unit, appear in the same chemical shift range. Therefore, the NMR analysis was repeated using methanol-d_4 at 300 K (600 MHz) as solvent. In this way, it was possible to distinguish H4*u* (*δ* 4.26) from H3*t* (*δ* 4.19), but the weak intensity of the correlation between H4*u* and both B-ring protons did not allow a definite determination. On the other hand, NOE interactions between H6*u* and H2′*t* as well as H6′*t* verified the 4→8 linkage. Once more, long-range $^{1}H-^{13}C$ couplings were observed in the HMBC between H4*u* and C7*t* (*δ* 151.0), C8a*t* (*δ* 150.1), and C8*t* (*δ* 105.5), confirming the 4→8 linkage. Furthermore, H6*t* correlated with C5*t* (*δ* 154.9) and C7*t* (*δ* 151.0).[36,37] Additionally, a correlation from H2*t* through the chroman-oxygen was observed to C8*t* and confirmed the C4*u*−C8*t* linkage.[38] If the two flavan-3-ol units were connected C4*u*−C6t, correlations should have been found between H4*u* and C7*t* (*δ* 151.0) as well as C6*t* (*δ* 105.5; instead of *δ* 96) and also to C5*t* (*δ* 154.9) and H8*t* with C8a*t* (*δ* 150.1), which was not the case.[36,37] Hence, the flavan-3-ol units have 2*uβ*→O7*t*, 4*uβ*→8*t* linkages. Key CH long-range correlations and NOE interactions of EGC-(2*β*→O7, 4*β*→8)-C are depicted in Figure 3.

The 3,4-*trans* or 3,4-*cis* configuration of the C-ring from the upper unit in 4→8-linked A-type proanthocyanidins could not be determined from the respective coupling constants, but was determined from NOE interactions. H3*u* showed a selective NOE effect to H6*t*. This correlation indicated a 3,4-*trans* configuration of the C-ring (Figure 3) and is in accordance with data from ref 18.

Consequently, the structure of compound **A** was deduced as EGC-(2*β*→O7, 4*β*→8)-C, which is reported here as a new natural product.

Structure Elucidation of EGC-(2*β*→O5, 4*β*→6)-C (Compound B). An analysis similar to the above was used to determine the structure of EGC-(2*β*→O5, 4*β*→6)-C, and only relevant data are summarized here. All NMR spectra were recorded at 300 K (600 MHz, acetone-d_6).

The ^{1}H NMR spectrum showed a characteristic AB pattern of the heterocyclic C-ring protons for A-type proanthocyanidins (H3*u* *δ* 4.15, d; H4*u* *δ* 4.33, d) with a coupling constant of $J_{3,4}$ = 3−4 Hz. The meta-coupled doublets at *δ* 6.08 and 6.09 (each J = 2.3 Hz), an aromatic proton singlet (H8*t*) at *δ* 6.05, and the AMX and AA′ systems in the aromatic region *δ* 6.71−6.84 confirmed the A-type dimeric proanthocyanidin structure. Moreover, the ketal C2*u* atom at *δ* 100.3 was assigned unequivocally by the 2D NMR analysis.[20−22] Hence, the C−C bond was at position C4*u*. The presence of 2*β*→O5, 4*β*→6 was proven from ^{1}H through-space interactions of the germinal protons H4*t* to the respective signals of H2′*u* and H6′*u* (NOESY spectra).

The HMBC correlation of C2*u* (*δ* 100.3) to the H2′*u*/H6′*u* protons of the trihydroxylated B ring indicated the upper unit is (epi)gallocatechin, whereas long-range connectivities (HMBC) between C1′*t* (*δ* 131.9) and C2′*t* (*δ* 115.0) as well as C6′*t* (*δ* 119.9) and the proton H2*t* (*δ* 4.60) indicated a terminal (epi)catechin unit.

The presence of a doublet at *δ* 4.60 with a large coupling constant of 7.6 Hz established the relative 2,3-*trans* configuration of the terminal unit, and the ^{13}C signal at *δ* 82.7 confirmed this was (+)-catechin.[24] (−)-Epigallocatechin of the upper unit was determined by means of chemical shift C3*u* (*δ* 68.4, (+)-gallocatechin; *δ* 67.0, (−)-epigallocatechin in acetone-d_6 at room temperature).[30] Furthermore, the upper flavan-3-ol unit was evident from the typical signal at *δ* 4.15 (*δ* 3.97, (+)-gallocatechin; *δ* 4.19, (−)-epigallocatechin in acetone-d_6 at room temperature).[30] The coupling constant of $J_{3,4A}$ = 3.5 Hz indicated the 3,4-*trans* configuration of (−)-epigallocatechin.[30]

As a result of these observations, the structure of this compound was established as EGC-(2*β*→O5, 4*β*→6)-C, which is also reported here for the first time as a natural product.

Structure Elucidation by CD and Phloroglucinolysis. The absolute configuration at C4 and C2 atoms of these two

compounds was established by their CD spectra in methanol. The 2β−4β orientation and hence the 4*R* configuration depicted in Figure 1 was confirmed by the intense positive Cotton effect at 220−240 nm of the CD curve.[18,39−41] Direct determination of the absolute stereochemistries of the two compounds by X-ray analysis[42,43] was not possible as suitable crystalline material could not be produced.

However, the determination of the nature of the flavan-3-ol units and the interflavanoid linkage is possible by acid-catalyzed depolymerization in the presence of phloroglucinolysis[14] as the ether bonds in A-type compounds are stable under thiolytic or phloroglucinol degradation conditions.[22,23,32,37,44] Hence, the A-type compounds EGC-(2β→O7, 4β→8)-C and EGC-(2β→O5, 4β→6)-C were not hydrolyzed by acid cleavage in the presence of phloroglucinol.

On the basis of all these data (i.e., HPLC-ESI-MS^n, NMR, CD, and phloroglucinolysis) EGC-(2β→O7, 4β→8)-C and EGC-(2β→O5, 4β→6)-C were established as novel prodelphinidins. Only the related A-type prodelphinidins EGC-(2β→O7, 4β→8)-EC and (−)-EGC-(2β→O7, 4β→8)-(−)-C are already known in the literature.[18,33,45] To the best of our knowledge, compounds **A6** (EC-(2β→O7, 4β→6)-EC) and **A7** (EC-(2β→O5, 4β→6)-EC) are unique examples of 4β→6- as well as 2β→O5-linked dimeric A-type proanthocyanidins.[31,32] Furthermore, the occurrence of EGC-(2β→O7, 4β→8)-C and EGC-(2β→O5, 4β→6)-C in hazelnuts or hazelnut skins is described for the first time. LC-MS analysis of a native hazelnut skin filtrate (70% aqueous acetone solution after precipitation with ethanol/*n*-hexane (5:13, v/v)) verified their natural occurrence in hazelnut. These two dimeric A-type prodelphinidins are minor components of roasted hazelnut skins.

AUTHOR INFORMATION

Corresponding Author

*(P.W.) Phone: +49-531-391-7200. Fax: +49-531-391-7230. E-mail: p.winterhalter@tu-bs.de.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

EGC, (−)-epigallocatechin; EC, (−)-epicatechin; C, (+)-catechin; HPLC-PDA, high-performance liquid chromatography photodiode array; LSRCCC, low-speed rotary countercurrent chromatography; HPLC-ESI-MS^n, HPLC−electrospray ionization multiple mass spectrometry; HR-ESI-MS, high-resolution ESI-MS; CD, circular dichroism; NMR, nuclear magnetic resonance

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