DOI:10.1021/jf101023e

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Dimeric Procyanidins: Screening for B1 to B8 and Semisynthetic Preparation of B3, B4, B6, and B8 from a Polymeric Procyanidin Fraction of White Willow Bark (*Salix alba*)

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

[†]Institute of Food Chemistry, Technische Universität Braunschweig, Schleinitzstrasse 20, 38106 Braunschweig, Germany, and [‡]Helmholtz Centre for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany

Fifty-seven samples have been analyzed with regard to the occurrence of dimeric procyanidins B1−B8 as well as the composition of polymeric procyanidins. Fifty-two samples were found to contain polymeric procyanidins. In most of the samples, (−)-epicatechin was the predominant unit present. In white willow bark (*Salix alba*), however, large amounts of (+)-catechin (81.0%) were determined by means of phloroglucinolysis. White willow bark has therefore been used for the semisynthetic formation of dimeric procyanidins B3 [(+)-C-4α → 8-(+)-C)], B4 [(+)-C-4α → 8-(−)-EC)], B6 [(+)-C-4α → 6-(+)-C)], and B8 [(+)-C-4α → 6-(−)-EC)]. The reaction mixtures of the semisynthesis were successfully fractionated with high-speed countercurrent chromatography (HSCCC), and dimeric procyanidins B3, B4, B6, and B8 were obtained on a preparative scale.

KEYWORDS: White willow bark (*Salix alba*); dimeric procyanidins; polymeric procyanidins; phloroglucinolysis; high-speed countercurrent chromatography; NMR

INTRODUCTION

Proanthocyanidins, also known as condensed tannins, are widely found as secondary metabolites in plants. They are present in fruits, barks, leaves and seeds of many plants. Proanthocyanidins are not only responsible for the bitter taste and astringent sensation of beverages and foods, for example tea, wine, and fruit juices, but are equally important for the color of food (*1*,*2*). Three common flavan-3-ols, which differ in their hydroxylation patterns, are found in proanthocyanidins. The procyanidins consist exclusively of (+)-catechin and/or (−)-epicatechin subunits. Propelargonidins and prodelphinidins, which are less abundant in nature compared to procyanidins, consist of (epi)afzelechin and (epi)gallocatechin subunits, respectively. In the so-called B-type procyanidins linkages between C_4 of the upper unit and C_8 of the lower unit predominate, while less abundant are $C_4 \rightarrow C_6$ linkages. The chemical structures of the four dimeric procyanidins B3, B4, B6, and B8 are shown in **Figure 1**. The flavan-3-ols can also be doubly linked by an additional ether bond between C_2 of the upper unit and the oxygen at C_7 or C_5 of the lower unit (so-called A-type procyanidins) (*3*). The low molecular weight procyanidins are usually present in plant tissue in relatively low concentrations compared to that of higher oligomers and polymers (degree of polymerization > 10) (*4*). They have been shown to evoke a number of biological responses such as antiallergic activity (*5*−*7*), anticaries activity (*8*), antihypertensive activity (*9*), and antioxidative activity (*10*−*13*).

Proanthocyanidins are the most abundant polyphenols in plants after lignins and they may comprise up to 50% in several barks (*14*). Until now, only a few foods have been studied for their proanthocyanidin content. The detailed proanthocyanidin profiles and compositions have already been determined for more than 40 common food sources (*15*−*17*). The foods with the highest levels of total proanthocyanidins were, in decreasing order, ground cinnamon, sorghum (early sumac bran), grape seed, unsweetened baking chocolate, raw pinto beans, chokeberries, red kidney beans, hazelnuts and pecan nuts (*16*, *17*). The distribution and the structural features of proanthocyanidins in many other food materials are still unknown. Furthermore, Arts et al. (*18*, *19*) quantified several fruits, vegetables, processed foods, such as chocolate, tea, wine and fruit juices for their content of catechins, including (+)-catechin, (−)-epicatechin, (+)-gallocatechin (GC), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin gallate (EGCG). (+)-Catechin and (−)-epicatechin were the most common catechins detected in foods. Despite these studies, data concerning the occurrence of dimeric procyanidins in foods is still scarce.

In the present study, a reversed-phase HPLC-ESI/MS/MS and/or HPLC-PDA method has been used for the first time to determine the presence of eight B-type procyanidin dimers B1−B8 in 57 samples and this has been complemented by compositional analysis of the polymeric procyanidins using phloroglucinolysis. To provide adequate amounts of pure dimeric

*Author to whom correspondence should be addressed. Telephone: +49-531-391-7200. Fax: +49-531-391-7230. E-mail: p.winterhalter@tu-bs.de.

 Published on Web 06/09/2010

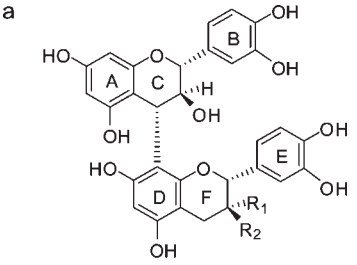

Figure 1. Structures of dimeric procyanidins of the B-type: (**a**) 4α → 8 linked dimeric procyanidins B3 and B4; (**b**) 4α → 6 linked dimeric procyanidins B6 and B8.

procyanidins, we have developed a semisynthetic preparation procedure (*20*, *21*). In a previous paper, we have described the successful semisynthetic preparation of dimeric procyanidins B1 [(−)-EC-4*β* → 8-(+)-C)], B2 [(−)-EC-4*β* → 8-(−)-EC)], B5 [(−)-EC-4*β* → 6-(−)-EC)], and B7 [(−)-EC-4*β* → 6-(+)-C)] from a polymeric fraction of black chokeberry (*Aronia melanocarpa*), which consisted almost exclusively of (−)-epicatechin units (*20*). A strategy for the semisynthesis of the remaining dimeric procyanidins B3 [(+)-C-4α → 8-(+)-C)], B4 [(+)-C-4α → 8-(−)-EC)], B6 [(+)-C-4α → 6-(+)-C)], and B8 [(+)-C-4α → 6-(−)-EC)] was not attempted. In this study, we present a semisynthetic route for the preparation of these dimeric procyanidins from a polymeric fraction of white willow bark (*Salix alba*). As large amounts of (+)-catechin were detected in the polymeric procyanidin fraction of white willow bark, this was chosen as starting material for the semisynthetic preparation of the remaining B-type dimeric procyanidins B3, B4, B6, and B8.

MATERIAL AND METHODS

Chemicals. (−)-Epicatechin, p.a. (Sigma, Steinheim, Germany), (+)-catechin-hydrate, ≥98% (Sigma), sodium acetate (anhydrous), p.a. (Merck, Darmstadt, Germany); sodium hydrogen carbonate, p.a. (Merck); phloroglucinol, p.a. (Merck); hydrochloric acid, 37% (Riedel-de-Haën, Seelze, Germany); ascorbic acid, pure (Merck); methanol, p.a. (Fisher Scientific, Loughborough, U.K.); acetonitrile, HPLC quality (Sigma, Steinheim, Germany); acetic acid, HPLC quality (Mallinckrodt Baker B.V., Deventer, The Netherlands); water (deionized, Nanopure); and acetone-d_6, 99,8% (Deutero GmbH, Kastellaun, Germany). Solvents used for High-speed countercurrent chromatography (HSCCC) and solvent precipitation were as follows: ethyl acetate (Acros Organics, Geel, Belgium); methanol (distilled, industrial quality); *n*-hexane (distilled, industrial quality); water (deionized, Nanopure); ethanol (distilled, industrial quality); 2-propanol, p.a. (Sigma); and acetone (distilled, industrial quality).

Authentic specimens of dimeric procyanidins B1, B2, B3, B4, B5, B6, B7, and B8 were prepared in our laboratory (*20*, *21*).

Analyzed Samples. Fruits, such as black chokeberry (*Aronia melanocarpa*), strawberry, pomegranate, peach, quince, and rose hip, were freeze-dried and ground into powder. Fifteen dried plant materials such as ginkgo, ribwort plantain, cinchona bark, quickthorn, rose hip seed coat, walnut peel, ladies mantle, silverweed, barley seed, rhododendron leaves, white willow leaves, white willow bark, cinnamon bark, hop, and almond peel (**Table 1**) obtained from Galke GmbH (Gittelde, Germany), were ground without freeze-drying. *Mimosa tenuiflora* bark (Spanish: tepezcohuite) and *Cyathea divergens* K. bark (Spanish: palo de vibora) were obtained from a local market in Guadalajara (Mexiko). Black rice was purchased from a local market in China.

Sixteen different grape seed extracts and grape polyphenol extracts (*Vitis vinifera* L.), goji berry extract, green tea extract, pine bark extract, as well as apple pomace extract were obtained from Breko GmbH (Bremen, Germany). Lingonberry leaves (*Vaccinium vitis-idea*), sessile oak leaves (*Quercus petraea*), sainfoin (*Onobrychis viciifolia*), and spruce needles (*Picea abies*) were harvested in June 2009 from the botanical garden of the TU Braunschweig (Braunschweig, Germany), freeze-dried and ground into a fine powder. *Croton lechleri* bark latex (sangre de drago) was purchased from BuenaNatura (Wiesloch, Germany).

Extraction. All plant tissues, except extracts and some foods, were defatted three times with *n*-hexane and subsequently extracted three times with 70% aqueous acetone solution. The acetone extracts were evaporated and freeze-dried. Eleven grams of this extract could be obtained from approximately 100 g of milled white willow bark (*Salix alba*).

Solvent Precipitation. Three grams of the freeze-dried 70% aqueous acetone extract of white willow bark were stirred for 1 h in 150 mL of ethanol and the insoluble residues were removed by filtration. Then 600 mL of *n*-hexane was slowly added dropwise to the solution (10 mL/min). The precipitate obtained was filtered, dissolved in water and lyophilized.

Phloroglucinolysis. Analysis was carried out according to method of Kennedy and Jones (*22*).

The aqueous acetone extracts of the different samples were subjected to phloroglucinolysis in concentrations of 0.4 to 1.7 g/L depending on their polymeric content. These analyses were performed in duplicate.

For the quantification of the extension ((+)-catechin-(4α → 2)-phloroglucinol, (−)-epicatechin-(4*β* → 2)-phloroglucinol) and terminal ((+)-catechin, (−)-epicatechin) units in polymeric procyanidin fractions with HPLC-PDA at 280 nm, calibration curves were used in a range of 2 to 500 mg/L. All reference compounds were dissolved in Nanopure water. Authentic specimen of phloroglucinol adducts ((+)-catechin-(4α → 2)-phloroglucinol, (−)-epicatechin-(4*β* → 2)-phloroglucinol, (−)-epicatechin-3-*O*-galloyl-(4*β* → 2)-phloroglucinol) as well as (−)-epicatechin-3-*O*-gallate were obtained by high-speed countercurrent chromatography (HSCCC) according to the method of Köhler and Winterhalter (*23*). HPLC conditions are the same as described in ref *20*.

Analysis of Isolated Dimeric Procyanidins. For partial cleavage of the interflavanoid bond, the reaction was carried out at 30 °C for 5 min and for complete depolymerization at 50 °C for 5 min, respectively. The reaction was terminated by adding 500 *μ*L sodium acetate solution (40 mM). For further information see Phloroglucinolysis.

Optimization of Reaction Conditions. Optimization of the ratio of substrates (i.e., white willow bark precipitate and the nucleophiles (+)-catechin or (−)-epicatechin), temperature, and reaction time were carried out by analogy with the procedure published in a previous study (*20*).

Semisynthetic Formation of Dimeric Procyanidins. Eight hundred milligrams of (+)-catechin or (−)-epicatechin and 400 mg of white willow bark precipitate (see above solvent precipitation) were used for semisynthesis. The precipitate and the respective flavan-3-ol were dissolved in 0.1 N methanolic HCl (50 mL) and the reaction was carried out at 30 °C for 20 min in a water bath. The reaction mixture was neutralized with 0.5 N sodium hydrogen carbonate solution (approximately 8 mL). The organic solvent was evaporated and the residual aqueous solution freeze-dried. Approximately 1.5 g of reaction mixtures were obtained after lyophilization.

HPLC Photodiode Array (PDA) Analysis. A HPLC system from Jasco (Gross-Umstadt, Germany), with a PU-2080 plus pump combined with a DG-2080−53 three-line-degasser and LG 2080−02 ternary gradient

Table 1. Flavan-3-ol Monomer and Dimeric Procyanidin Constituents in Different Samples

no.	sample[a]	scientific or brand name	C	EC	B1	B2	B3	B4	B5	B6	B7	B8
1	black chokeberry	*Aronia melanocarpa*		x	x	x			x		x	
2	strawberry	*Fragaria* L.	x	x	x		x			x	x	
3	pomegranate	*Punica granatum* L.	x	x	x		x				x	x
4	peach	*Prunus persica* L.	x		x						x	
5	quince	*Cydonia oblonga* Mill.		x		x			x			
6	rose hip	*Rosa canina*	x		x		x			x	x	
7	cocoa bean, fermented, unroasted	*Theobroma cacao* L., sem. ferm. intorr.	x	x		x			x			
8	black rice seed[b]	*Oryza sativa,* semen										
9	raisin pomace[c]	*Racemus, vinacea*										
10	hazelnut seed coat	*Corylus, cort.*	x	x	x	x	x	x		x	x	
11	chestnut peel	*Castanea, cort.*	x	x		x					x	
12	peanut peel	*Arachis hypogaea* L., *cort.*	x	x		x	x	x	x		x	
		Commercial Dried Samples by Galke GmbH										
13	ginkgo leaves, uncut[b]	*Ginkgo biloba Fol. Tot.*	x									
14	ribwort plantain, cut[b]	*Plantaginis lanc. Hb.conc.*										
15	cinchona bark, cut	*Chinae Cortex conc.*		x		x			x	x		
16	quickthorn leaves with flowers, cut	*Crataegus oxyac. Fol. c. Flor. conc.*		x		x			x			
17	rose hip seed coat, sliced	*Cynosbati Fruct. s. sem. conc.*	x	x	x		x				x	
18	walnut peel, cut[b]	*Juglandis nuc. cort. conc.*										
19	ladies mantle, cut	*Alchemillae vulg. Hb. conc.*	x				x			x		
20	silverweed, cut	*Anserinae Hb. conc.*	x		x		x			x	x	
21	barley seed, whole	*Hordei Fruct. tot.*	x				x			x		
22	rhododendron leaves, cut	*Rhododendri Fol. conc.*	x	x	x	x	x	x			x	
23	willow leaves, cut	*Salicis alba Fol. conc.*	x		x		x			x	x	
24	white willow bark, cut	*Salicis Cort. Alba conc.*	x		x		x			x	x	
25	cinnamon bark Ceylon, cut	*Cinnamomi Ceyl. Cort. conc.*		x		x			x			
26	hops, whole	*Humuli lupuli Strobuli tot.*	x	x	x	x	x	x		x	x	
27	almond peel, cut	*Amygdalae dulc. Cort. Conc.*	x	x	x	x	x		x		x	
		Commercial Dried Samples										
28	tepezcohuite bark	*Mimosa tenuiflora, cort.*	x								x	
29	Palo de vibora bark	*Cyathea divergens Kunze, cort.*				x						
30	coco bark[c]	*Cocos nucifera, cort.*										
31	Pine bark	*Pinus pinea, cort.*	x	x	x		x			x	x	x
32	sangre de drago bark latex	*Croton lechleri, cort. lat.*	x	x	x	x		x	x			
33	grape-vine	*Vitis vinifera* L.	x	x	x	x	x	x	x	x	x	
		Commercial Extracts										
		Grape Polyphenol Extracts (*Vitis vinifera* L.)										
34	grape polyphenol extract P20[c]											
35	grape polyphenol extract P50[c]											
36	grape polyphenol extract P70[c]											
37	grape polyphenol extract P80 white[c]											
38	grape polyphenol extract P80 red[c]											
39	grape polyphenol extract P90 red[c]											
40	grapeskin-powder red (GSP)[c]											
		Grape Seed Extracts (*Vitis vinifera* L.)										
41	grape seed extract P100[c]											
42	grape seed extract P95[c]											
43	grape seed extract OPC 30		x	x	x	x	x	x	x	x	x	x
44	grape seed extract OPC 40 (2006)		x	x	x	x	x	x	x	x	x	x
45	grape seed extract OPC 40 (2006)		x	x	x	x	x	x	x	x	x	x
46	grape seed extract OPC 40 (2007)		x	x	x	x	x	x	x	x	x	x
47	grape seed extract OPC 40 (2008)		x	x	x	x	x	x	x	x	x	x
48	grape seed P.E. extract[c]											
49	grape seed 95% extract[c]											
50	goji berry extract[b]	*Lycium barbarum, decoct.*										
51	green tea extract	*Camellia sinensis, decoct.*	x	x		x		x			x	
52	Pine bark extract	*Pinus larix* L., *cort., decoct.*	x	x	x	x	x	x	x		x	x
53	apple pomace extract	*Malus, vinacea*		x		x						
		Leaves										
54	lingonberry	*Vaccinium vitis-idea*	x	x	x	x	x	x	x	x	x	
55	sessile oak	*Quercus petraea*	x		x		x			x	x	
56	sainfoin[b]	*Onobrychis viciifolia*										
57	spruce needles	*Picea abies*	x	x	x		x			x	x	

[a] Commercial extract or 70% aqueous acetone (v/v) extract. [b] No dimeric procyanidins detected. [c] Not analyzed by HPLC-ESI/MS//MS.

unit, and MD-2010 plus DAD were used. HPLC conditions are the same as described in (*20*).

HPLC-ESI/MS/MS Analysis. Chromatographic analyses were performed on an Agilent 1100 HPLC system (Waldbronn, Germany) equipped with an 1200 autosampler and an 1100 HPLC pump. The HPLC was also coupled to the Esquire HPLC-ESI/MS system from Bruker GmbH (Bremen, Germany). HP ChemStation was used for data collection. MS parameters: negative mode; capillary, +3000 V; end plate, −500 V; capillary exit, −105 V; dry gas was nitrogen, 325 °C; gas flow, 10 L/min; and nebulizer pressure, 40 psi. During the chromatographic run mass spectra of the effluent were recorded from m/z 50 to 2200. HPLC conditions were the same as described earlier (*20*).

Preparative HPLC. A HPLC system from Knauer (Berlin, Germany) was used consisting of a Smartline 1000 HPLC pump, Smartline Manager 5000 solvent organizer and degasser, Wellchrom K-2600 UV detector, Rheodyne 7125 injector (200 μL), and ChromGate V3.1.7 software.

The following preparative HPLC columns were used: (a) Hypersil ODS C-18 5 μm, 250 × 16 mm i.d. (M & W Chromatographietechnik GmbH, Berlin, Germany) and (b) Aqua 5 μ C-18, 125 Å, 250 × 21.2 mm i.d. (Phenomenex, Aschaffenburg, Germany). Column (a) was used for the isolation of dimeric procyanidin B6 and column b) for dimeric procyanidin B8. Water (solvent A) and acetonitrile (solvent B) were used as solvent systems.

Gradient 1 (for dimeric procyanidin B6): initial 10% B in 40 min to 30% B; gradient 2 (for dimeric procyanidin B8): initial 10% B in 50 min to 35% B. The flow rate was 6.0 mL/min.

Nuclear Magnetic Resonance (NMR) Spectroscopy. One-dimensional ^{1}H NMR and two-dimensional ^{1}H−^{1}H correlation spectroscopy (COSY), J-resolved spectroscopy and rotating frame nuclear Overhauser enhancement spectroscopy (ROESY) experiments were recorded at 240 or 260 K on a Bruker ARX400 NMR spectrometer equipped with a variable temperature unit B VT-2000 (Rheinstetten, Germany) locked to the deuterium resonance of the solvent, acetone-d_6. Chemical shifts are given in ppm relative to the residual proton signals of the solvent (2.05 ppm) and coupling constants are given in Hz.

HSCCC. The preparative HSCCC instrument used in the present study was a multilayer coil planet centrifuge model CCC-1000 (Pharma-Tech Research Corp.; Baltimore, MD, USA) equipped with three preparative coils connected in series (total volume: 800 mL). The separation was carried out at 1000 rpm with flow rates of 2.7 mL/min. The mobile and stationary phase was delivered with a Biotronik BT 3020 HPLC pump (Jasco, Grossumstadt, Germany). The elution mode was head to tail. All samples were dissolved in a 1:1 mixture of upper and lower phase. A manual sample injection valve equipped with a 20 mL loop was used to introduce the sample into the coil system. The effluent stream was monitored by UV detection at 280 nm with a Knauer UV/vis detector (Berlin, Germany) and recorded using a BBC Goerz SE 120 plotter (3 cm/h). The effluent stream was collected in 12 mL fractions by use of a Super Frac fraction collector (Pharmacia, LKB Super Frac fraction collector). Two phase solvent systems composed of ethyl acetate/isopropanol/water (20:2:20, v/v/v) were used for the fractionation of dimeric procyanidin B3, ethyl acetate/isopropanol/water (20:1:20, v/v/v) for B4, and *n*-hexane/ethyl acetate/methanol/water (1.2:10:1.2:10, v/v/v/v) for B6 and B8, respectively.

RESULTS AND DISCUSSION

Screening of Dimeric Procyanidins. In this study, 57 different samples were screened for the presence of dimeric procyanidins B1−B8. Flavan-3-ol monomers and procyanidin dimers were identified by comparison with the retention times and typical UV data of authentic reference standards as well as their mass spectra (*24*, *25*). **Table 1** shows the distribution of flavan-3-ol monomers and dimeric procyanidins B1−B8 in different samples. We have selected these different samples, due to their astringent sensation, their commercial availability or the known occurrence of proanthocyanidins. In a recent publication we have reported the semisynthetic formation of dimeric procyanidins B1, B2, B5, and B7 (*20*). A slight forward approach for the synthesis of the remaining dimeric procyanidins B3, B4, B6, and B8, however, is still missing. Due to this fact, we especially have payed attention to the occurrence of B3, B4, B6, and B8 in nature. Dimeric procyanidin B6 was found in 14 samples such as strawberries, rose hip, hazelnut seed coat, cinchona bark, ladies mantle, silverweed, barley seed, white willow leaves, white willow bark,

Table 2. Structural Composition of the Extension Units of 57 Samples, Determined by HPLC-PDA at 280 nm Following Phloroglucinolysis Depolymerization

		proportion of extension units (mg/g)	
no.	sample	C-Ph	EC-Ph
1	black chokeberry	11.4	634.2
2	strawberry	5.4	7.7
3	pomegranate	0	0
4	peach	5.5	106.2
5	quince	8.4	246.4
6	rose hip	28.9	51.1
7	cocoa bean	0	192.3
8	black rice	0	0
9	raisin pomace	43.7	66.3
10	hazelnut seed coat	121.1	246.6
11	chestnut peel	0	214.9
12	peanut peel	7.2	38.2
13	ginkgo	0	7.4
14	ribwort plantain	2.4	1.4
15	cinchona bark	2.7	40.8
16	quickthorn	4.2	101.2
17	rose hip seed coat	4.6	5.2
18	walnut peel	2.7	0.9
19	ladies mantle	15.7	9.8
20	silverweed	12.0	6.3
21	barley seed	12.2	0
22	rhododendron leaves	16.2	63.5
23	white willow leaves	93.0	10.4
24	white willow bark	134.4	31.5
25	cinnamon bark	5.6	172.6
26	hops	10.2	33.7
27	almond peel	6.8	85.5
28	tepezcohuite bark	0	0
29	palo de vibora bark	0	144.9
30	coco bark	0	26.6
31	pine bark	43.1	47.6
32	sangre de drago bark latex	34.3	65.9
33	grape-vine	22.4	90.0
34	grape polyphenol extract P20	18.7	84.1
35	grape polyphenol extract P50	10.7	40.6
36	grape polyphenol extract P70	22.9	220.2
37	grape polyphenol extract P80 white	28.3	149.8
38	grape polyphenol extract P80 red	15.7	76.6
39	grape polyphenol extract P90 red	43.4	249.9
40	grapeskin-powder red (GSP)	9.8	40.9
41	grape seed extract P100	67.4	418.6
42	grape seed extract P95	66.0	391.6
43	grape seed extract OPC 30	70.7	303.2
44	grape seed extract OPC 40 (2006)	60.8	242.8
45	grape seed extract OPC 40 (2006)	71.8	339.9
46	grape seed extract OPC 40 (2007)	73.4	333.4
47	grape seed extract OPC 40 (2008)	64.4	308.4
48	grape seed extract P.E.[a]	56.2	405.3
49	grape seed extract 95%[a]	61.2	404.9
50	goji berry extract	0	0
51	green tea extract	0	0
52	pine bark extract	41.5	282.0
53	apple pomace extract	0	61.4
54	lingonberry leaves	36.2	180.8
55	sessile oak leaves	9.8	111.4
56	sainfoin	2.2	2.5
57	spruce needles	5.9	105.3

[a] Not analyzed by HPLC-ESI/MS//MS.

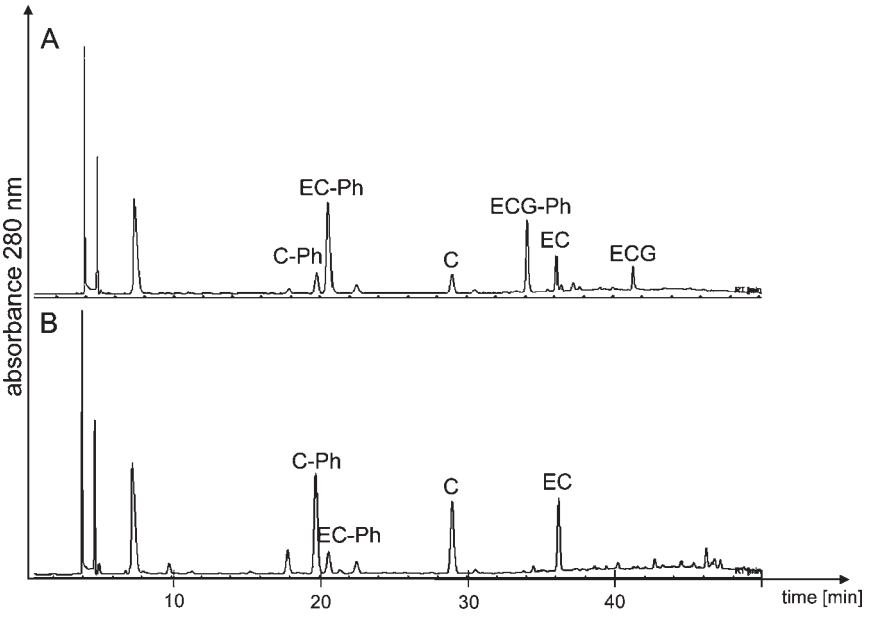

Figure 2. HPLC-PDA chromatograms of depolymerized phloroglucinolysis products: (**A**) grape seed extract; (**B**) 70% aqueous acetone (v/v) extract of white willow bark. (C-Ph: (+)-catechin-(4α → 2)-phloroglucinol. EC-Ph: (−)-epicatechin-(4β → 2)-phloroglucinol. C: (+)-catechin. ECG-Ph: (−)-epicatechin-3-*O*-galloyl-(4β → 2)-phloroglucinol. EC: (−)-epicatechin. ECG: (−)-epicatechin-3-*O*-gallate.)

hops, grape-vine, lingonberry leaves, sessile oak leaves, and spruce needles. Moreover, dimeric procyanidin B8 was detected only in pomegranate and commercial pine bark extract (*Pinus larix* L.). In commercial dried pine bark (*Pinus pinea*) and five analyzed commercial grape seed extracts both dimeric procyanidins B6 and B8 were identified. According to HPLC-ESI/MS/MS analysis, no flavan-3-ol monomers and dimeric procyanidins were present in black rice, ribwort plantain, walnut peel, commercially goji berry extract and sainfoin. The low concentration of dimeric procyanidins B6 and B8, as well as B3 and B4 (except grape seed extracts) did not allow a subsequent preparative isolation.

Phloroglucinolysis. Due to the absence of detailed information regarding the composition of oligomeric and polymeric procyanidins, phloroglucinolysis was applied in this work to determine the extension and terminal units (data not shown) of procyanidins in the 57 samples (i.e., fruits, leaves, barks). Forty-six of these samples were found to contain (+)-catechin in the extension units. The composition of the extension units is shown in **Table 2**. The existence and composition of polymeric procyanidins in most of the analyzed sources have not been described earlier. As examples, the chromatograms of depolymerized phloroglucinolysis products of grape seed extract and white willow bark are shown in **Figure 2**. During phloroglucinolysis of white willow bark, (+)-catechin and (−)-epicatechin in the extension units of polymeric procyanidins lead to (+)-catechin-(4α → 2)-phloroglucinol and (−)-epicatechin-(4β → 2)-phloroglucinol, and the terminal units liberate (+)-catechin or (−)-epicatechin. In the present paper, we only focused on polymeric proanthocyanidins which are composed of (+)-catechin and/or (−)-epicatechin in the extension units.

The common extension unit determined in procyanidins is (−)-epicatechin which agrees with the data published by Foo and Porter (*26*). Our results also show that the polymeric proanthocyanidin fractions of the studied ribwort plantain, walnut peel, ladies mantle, silverweed, barley seed, white willow bark, and white willow leaves are predominantly composed of (+)-catechin units. In white willow barks and leaves (+)-catechin and (−)-epicatechin occur as monomers and, in addition, as terminal and extension units in the polymeric procyanidin fraction. The highest yield of (+)-catechin in the extension units was detected in white willow barks and white willow leaves. Because of the rather complex composition of white willow leaves, the bark was chosen for the semisynthetic preparation of dimeric B-type procyanidins which contain a (+)-catechin unit as extension group, that is, B3, B4, B6, and B8.

Depolymerization of Polymeric Procyanidins in White Willow Bark. For enrichment of oligomeric and polymeric procyanidins from a 70% aqueous acetone extract (v/v) of white willow bark, solvent precipitation with ethanol and *n*-hexane in a ratio of 1:4 was carried out. Oligomeric and polymeric procyanidins of white willow bark were enriched in the precipitate and the other compounds in the filtrate (**Figure 3**).

Our results of phloroglucinolysis revealed that polymeric procyanidins in the extension units consist of 81.0% (+)-catechin and 19.0% (−)-epicatechin. Consequently, white willow bark represents a good source for the formation of dimeric procyanidins with (+)-catechin in the upper unit by semisynthesis, that is, the dimers B3, B4, B6, and B8. The detailed mechanism of semisynthesis has been described earlier (*20*, *21*). Under acidic conditions the interflavanoid linkage of polymeric procyanidins are depolymerized and positively charged carbocations (extension units) and uncharged flavan-3-ol units as terminal units are obtained. The carbocations can then react with an excess of nucleophiles, such as (+)-catechin and (−)-epicatechin, to afford dimeric procyanidins.

Semisynthesis. As described earlier (*20*, *21*), the ratio of substrates (i.e., white willow bark precipitate and nucleophile), temperature, and reaction time, must be optimized (**Figure 4**) to increase the yield of dimeric procyanidins and to minimize the formation of byproduct such as chalcane flavan-3-ols (gambiriins). The graphs for (−)-epicatechin (**A**−**C**) and (+)-catechin as nucleophile (**D**−**F**) are very similar. The yield of dimeric procyanidins and also of chalcane flavan-3-ols increases in cases of higher concentrations of flavan-3-ol. A ratio of 1:2 or 1:3 of white willow bark precipitate: nucleophile was found to give the highest yield of the target compounds B3, B4, B6, and B8.

Formation of dimeric procyanidins declines with increasing temperature from 30 to 50 °C whereas the yield of gambiriins increases. The highest yield of dimeric procyanidins was

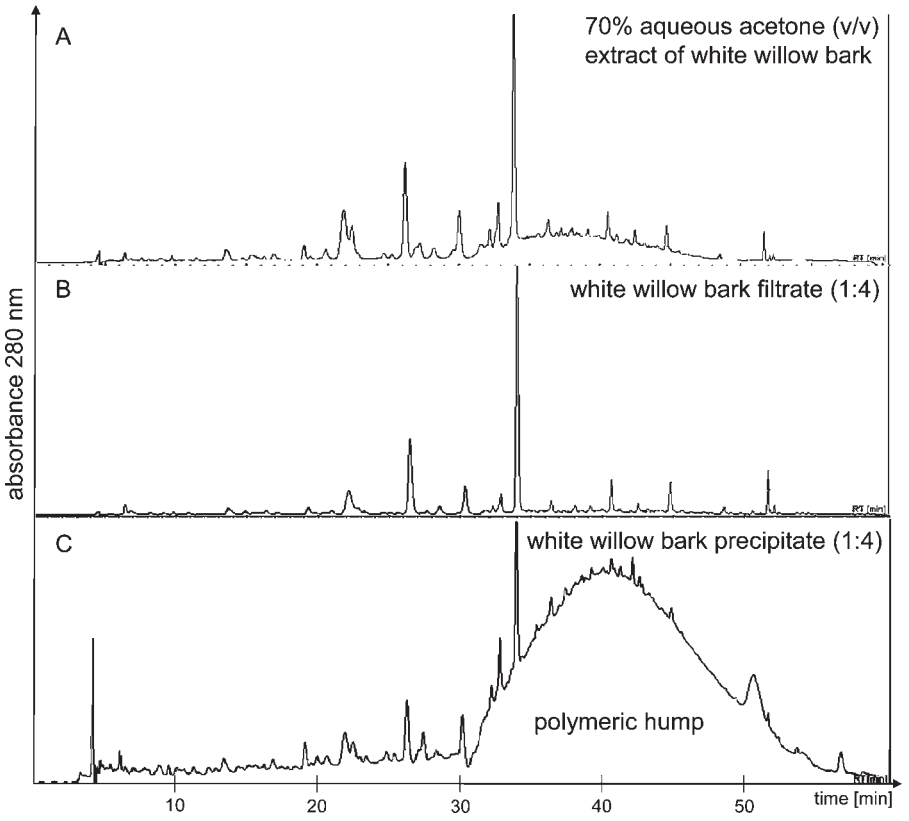

Figure 3. HPLC-PDA chromatograms of white willow bark before (**A**) and after (**B**, **C**) solvent precipitation.

determined at 30 °C. The reaction time was found to have a minor influence on the yield of dimeric procyanidins while the formation of gambiriins increases significantly with increasing reaction time from 10 to 30 min.

Under the optimized reaction parameters (i.e., substrate ratio of 1:2 polymeric procyanidin fraction and nucleophiles, temperature of 30 °C, and reaction time of 20 min) a maximum formation of dimeric procyanidins was observed. The rate of byproduct formation was in the range of 8.9% (in case of (−)-epicatechin as nucleophile) to 16.0% (in case of (+)-catechin as nucleophile).

Figure 5 shows the results of semisynthesis under optimized conditions. The HPLC-PDA chromatogram of the white willow bark precipitate before semisynthesis is displayed in **Figure 5A**. The polymeric procyanidins appear as a broad unresolved peak between 30 and 55 min. After semisynthesis with (+)-catechin or (−)-epicatechin, the polymeric procyanidins are depolymerized while the concentration of dimeric procyanidins increased (**Figure 5B** and **C**). Because of the composition of the polymeric procyanidins from white willow bark (about 81.0% of (+)-catechin in the extension unit) mainly dimeric procyanidins are formed during semisynthesis which contain (+)-catechin in the extension unit, that is, B3 (C-4→8-C), B4 (C-4→8-EC), B6 (C-4→6-C), and B8 (C-4 → 6-EC). Lower amounts of dimeric procyanidins with (−)-epicatechin in the extension unit, such as procyanidins B1 (EC-4→8-C), B2 (EC-4→8-EC), B5 (EC-4→6-EC), and B7 (EC-4→6-C), were also produced. The highest yield was obtained for 4→8 linked dimeric procyanidins with (+)-catechin in the extension unit (i.e., dimers B3 and B4).

HSCCC Separations. HSCCC separations of the reaction mixture from semisynthesis with white willow bark precipitate with (+)-catechin are shown in **Figure 6A** and **B** (CCC-1 and CCC-2). The solvent system was composed of ethyl acetate/isopropanol/water (20:2:20, v/v/v) and 1.3 g of sample was injected for the separation with HSCCC (CCC-1). Fraction 1 contained 373 mg of nonreacted polymeric procyanidins. From fraction 2, 26 mg of dimeric prodelphinidin (molecular ion $[M-H]^-$ at 593 m/z, purity = 66%) was obtained. Dimeric procyanidin B1 was present in fraction 3 (19 mg; 60%). Fraction 4 contained an epimerized dimeric procyanidin (9 mg; 74%). Fraction 5 consisted of dimeric procyanidin B3 (79 mg; 96%) and the last fraction 6 contained two unknown trimeric procyanidins. Subsequently, the coil fraction from CCC-1 was fractionated using a more lipophilic solvent system composed of *n*-hexane/ethyl acetate/methanol/water (1.2:10:1.2:10, v/v/v/v). The results are shown in **Figure 6B** (CCC-2). Polymeric procyanidins were recovered in fraction 1 (6 mg) and dimeric prodelphinidin (12 mg) was enriched in fraction 2. Fraction 3 consisted of 9 mg of gambiriin A1 (70%). Fraction 4 (34 mg) contained dimeric procyanidin B6 and B7 (24%; 61%). Fraction 5 was divided in two parts a and b, and both contained dimeric procyanidin B6 (3 mg; 74% and 7 mg; 61%, respectively). The excess of unreacted (+)-catechin was isolated in >98% purity from fraction 6 (567 mg).

The HSCCC separations of the reaction mixture of the semisynthesis with white willow bark precipitate and (−)-epicatechin are shown in **Figure 6C** and **D** (CCC-3 and CCC-4). The solvent system ethyl acetate/2-propanol/water (20:1:20, v/v/v) was used (*21*) and 1.3 g of sample were again injected for the separation with HSCCC (CCC-3). The main compound procyanidin B4 was enriched in fraction 5 of CCC-3 with a purity of 86% (73 mg). Polymeric procyanidins were accumulated in fraction 1 (356 mg) and fraction 2 (28 mg) contained an (epi)catechin/(epi)gallocatechin derivate with a molecular ion $[M-H]^-$ at 593 m/z. Fractions 3 and 4 contained the dimeric procyanidin B2 (18 mg; 9 mg).

The more lipophilic compounds that remained on the coil were separated (CCC-4, **Figure 6D**) with the same solvent system used for CCC-2. The dimeric procyanidin B8 and B5 were present in fraction 5 (13 mg; 65 and 33%, respectively). In addition, fraction

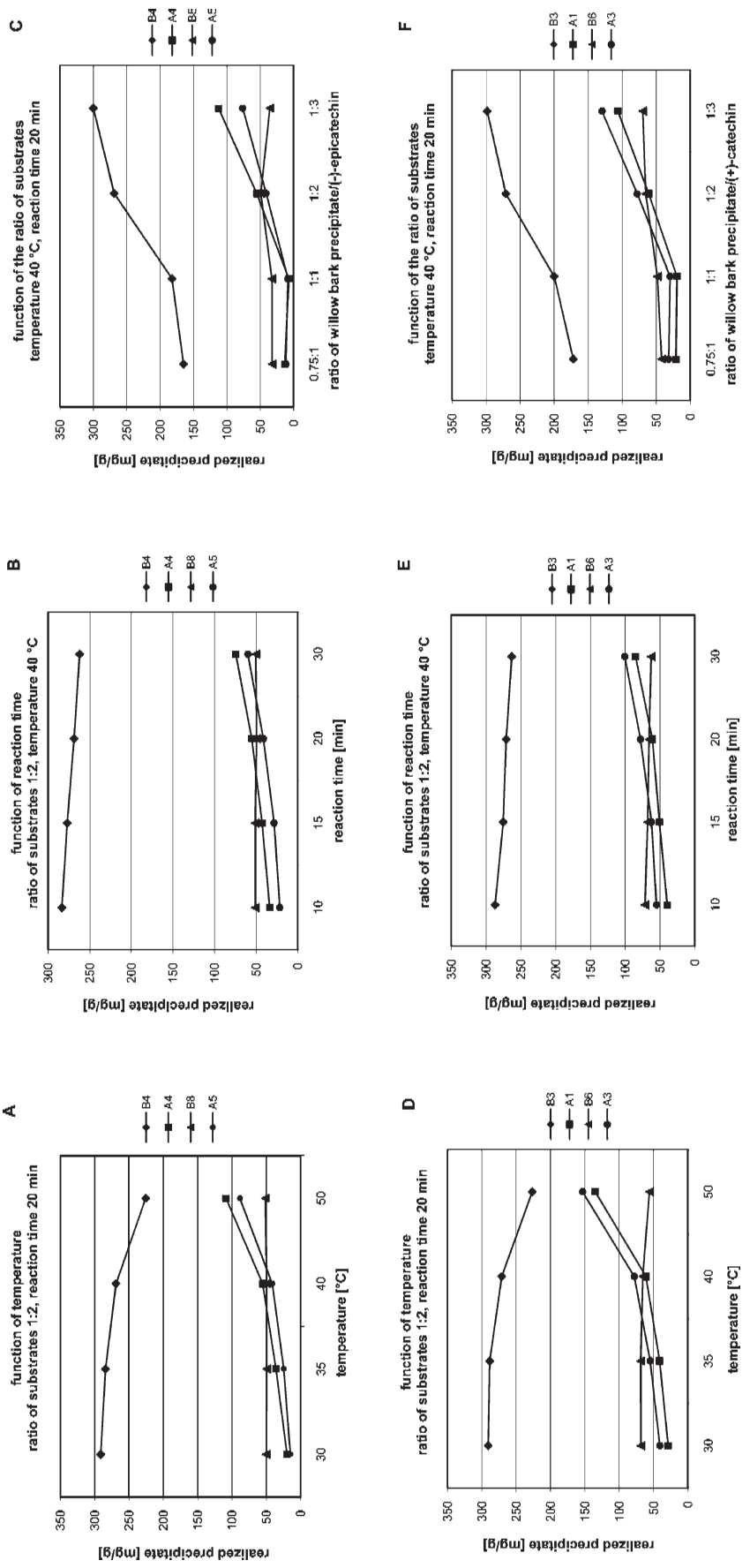

Figure 4. Optimization of reaction conditions for semisynthesis (**A**, **B**, **C**) with (−)-epicatechin and (**D**, **E**, **F**) with (+)-catechin. Product amounts were calculated as dimeric procyanidin B1 equivalents. B3, B4, B6 and B8 are dimeric procyanidins and A1, A3, A4 and A5 are gambiriins.

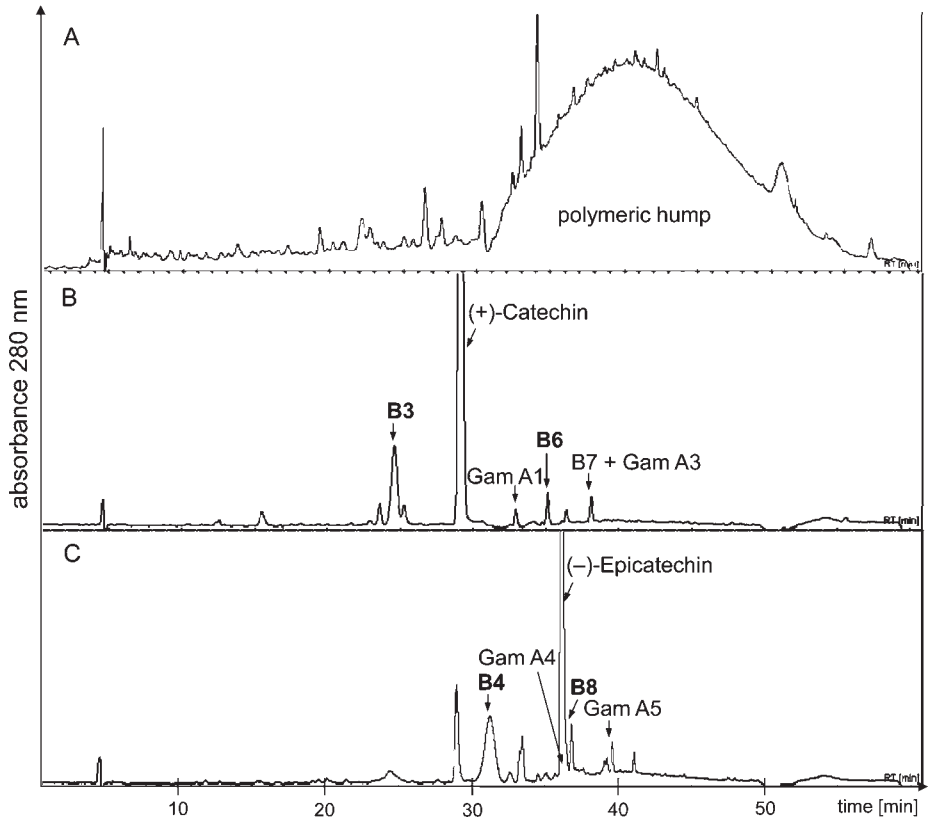

Figure 5. HPLC-PDA chromatograms of the white willow bark precipitate before semisynthesis (**A**) and after semisynthesis under optimized conditions (given in text) with (+)-catechin (**B**) or (−)-epicatechin (**C**) as nucleophile. (Gam, Gambiriin). B1, B2, B5, and B7 are likewise formed, but in smaller amounts (not indicated).

1 contained polymeric procyanidins (12 mg) and fraction 2 an unknown trimeric procyanidin (7 mg). Fraction 3 (5 mg) contained a mixture of dimeric prodelphinidin, gambiriin A4, and an (epi)catechin/(epi)afezelechin derivative with a molecular ion $[M - H]^-$ at 561 *m*/*z*. Gambiriin A4 was present in fraction 4 (9 mg; 63%) and the unreacted (−)-epicatechin was isolated from fraction 6 (538 mg; >98%). The last fraction contained (+)-catechin (40 mg; 87%).

Structure Elucidation of Dimeric Procyanidins B6 and B8. The isolated dimeric procyanidins B3, B4, B6, and B8 were finally purified by preparative HPLC. Their structures were characterized from 1D and 2D (COSY, J-resolved, and ROESY) NMR data. The interpretation of the spectra of dimeric procyanidins with (+)-catechin in the extension unit is complicated by the phenomena of rotational isomerism caused by slow rotation around the interflavanoid bond. This causes a broadening of the spectra at room temperature and prevents signal assignments. This was overcome by recording spectra at low temperature in acetone-d_6 as solvent, where nearly equal amounts of two rotamers were found for B6 and B8. Dimeric B-type procyanidins B3 and B4 were identified by comparison of their NMR and HPLC-MS data with the literature (*27*). The unambiguous structures of the dimeric procyanidins B6 and B8 follow from the NMR data reported in **Tables 3** and **4**, respectively. Correlations in the 2D COSY spectra and integration of the 1D spectra allowed identification of the number of protons involved in the various spin systems in both sets of rotamers of B6 and B8. The magnitude of the coupling constants in the different units were determined from the 1D spectra. In the case of B6 a 2D J-resolved spectrum was recorded after addition of a small amount of CD_3OD to cause exchange of the OH3 group and hence simplification of H3 in both the extension and terminal units. This spectrum allowed determination of the sum of the couplings of H2, H3, and H4 of the extension unit (**Table 3**), which were only compatible with an axial disposition of these protons and confirmed the presence of a (+)-catechin unit. The magnitude of the vicinal couplings determined for the terminal unit were also only compatible with a second (+)-catechin unit. Again for B8 the extension unit was identified as (+)-catechin, while the singlet observed for H2 and small magnitude of the vicinal couplings to both H4A and H4B indicated the terminal unit was composed of an (−)-epicatechin system.

The nature of the terminal units were confirmed by positive correlations in the 2D phase-sensitive ROESY where the (+)-catechin unit of B6 was characterized by through-space interactions between H2 and axial H4 and between H3 and equatorial H4, while the (−)-epicatechin system of B8 showed interactions between H2 and both H3 and axial H4, and H3 correlated with both H4 protons. Further positive correlations were observed between H2′ and H6′ of the B and E rings with the adjacent H2, H3, and OH3 of both compounds confirmed the equatorial disposition of these aromatic ring systems in B6 and B8.

Our observations for a considerable number of compounds indicate the inter-residue linkage can be determined from the presence or absence of characteristic signals in the 2D phase-sensitive ROESY associated with the aromatic ring system E of the terminal unit in the case of procyanidin dimers and of similar aromatic systems in the central units of larger polymers. Without exception 4 → 8 linkages always show positive correlations between H2′ (and H6′) of ring E of the terminal system with H4 of ring C of an adjacent system. No such correlations are observed for 4 → 6 linkages. Similar observations have been made for directly linked flavanol-anthocyanin dimers (*28*). In the case

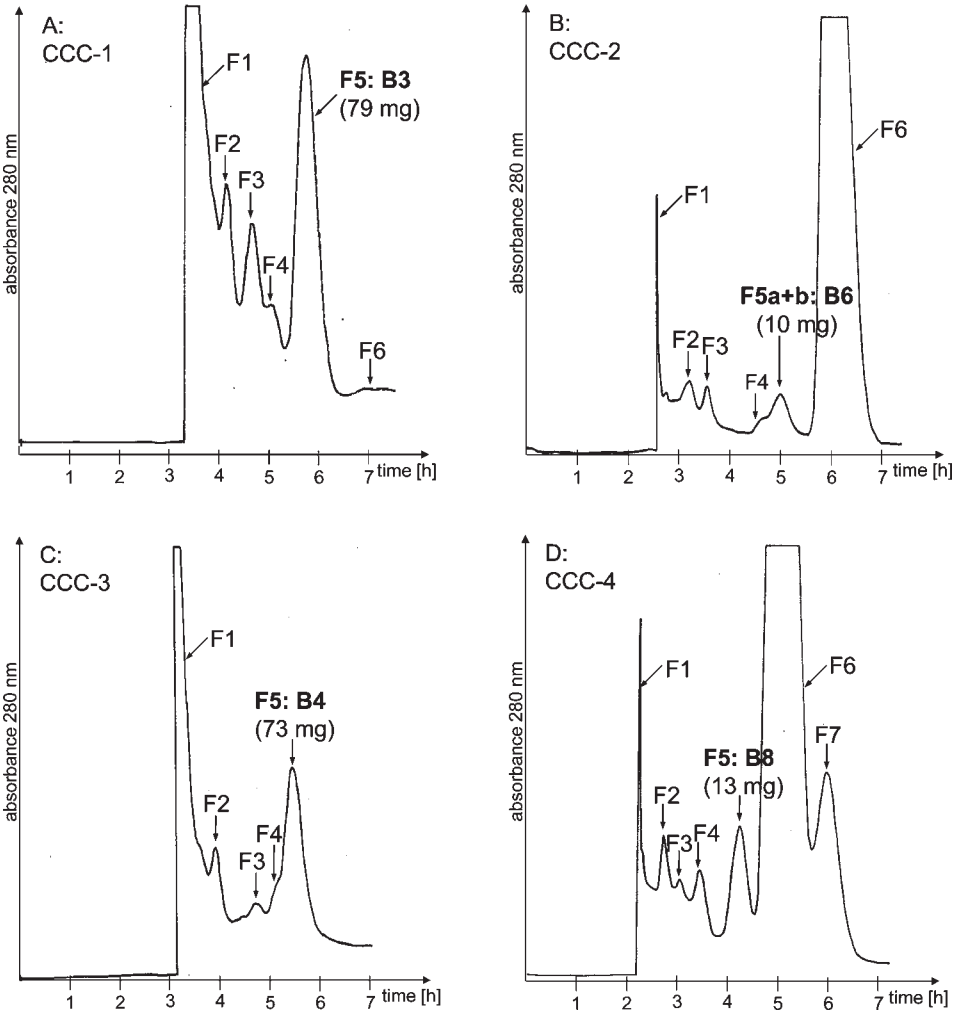

Figure 6. (**A**) CCC-1: HSCCC chromatogram of the reaction products of white willow bark precipitate and (+)-catechin. Injection of sample, 1.3 g; two phase solvent system: ethyl acetate/isopropanol/water (20:2:20, v/v/v); retention of the stationary phase (*R*s), 38.9%. (**B**) CCC-2: HSCCC chromatogram of the coil fraction of CCC-1, 681 mg sample; two phase solvent system: *n*-hexane/ethyl acetate/methanol/water (1.2:10:1.2:10, v/v/v/v); *R*s = 53.8%. (**C**) CCC-3: HSCCC chromatogram of the reaction products of white willow bark precipitate and (−)-epicatechin, 1.3 g sample; two phase solvent system: ethyl acetate/2-propanol/water (20:1:20, v/v/v); *R*s = 36.3%. (**D**) CCC-4: HSCCC chromatogram of the coil fraction of CCC-3, 655 mg sample; two phase solvent system: *n*-hexane/ethyl acetate/methanol/water (1.2:10:1.2:10, v/v/v/v); *R*s = 56.4%.

of B6 and B8, the absence of such correlations is a strong indication of 4 → 6 linkages and is in contrast to the positive correlations found for B3 and B4, the 4 → 8 linked isomers of B6 and B8.

A further phenomenon of importance is the observation of exchange peaks that appear as negative correlations in the 2D phase-sensitive ROESY spectra where each signal in one rotamer correlates with the same signal of the second isomer. Such observations confirm the doubling of signals observed at low temperature was indeed caused by rotational isomerism and not by the presence of two independent compounds of approximately equal concentration.

The structures of dimeric procyanidins B6 and B8 were confirmed by a complete depolymerization by phloroglucinolysis. Both compounds yielded (+)-catechin-(4α→2)-phloroglucinol indicating (+)-catechin in the extension unit and (+)-catechin as terminal unit for B6 and (−)-epicatechin as terminal unit for B8. Mild phloroglucinolysis allowed determination of the interflavanoid bond as 18.7% unreacted dimeric procyanidin B6 and 22.4% unreacted B8 remained after reaction indicating 4 → 6 linkages. In contrast, the 4 → 8 linkages of the dimeric procyanidins B3 and B4 followed from the lower amounts of unreacted material (7.3% unreacted B3 and < 1% unreacted B4) under the same conditions.

In conclusion, 57 different samples have been analyzed with regard to their dimeric and polymeric procyanidin composition. Polymeric procyanidins of white willow bark were found to contain the highest amount of (+)-catechin in the extension units. Hence, this natural product represents a good starting material for the semisynthetic formation of the dimeric procyanidins B3, B4, B6, and B8, which can be successfully isolated by HSCCC. The major products are procyanidins B3 and B4 which carry 4 → 8 linkages and afford purity levels of dimeric procyanidin B3 > 95%. At the same time lower amounts of the 4 → 6 linked dimeric procyanidins B6 and B8 are produced.

ACKNOWLEDGMENT

Different samples were kindly provided by Wolfgang Lörsch (Breko GmbH, Bremen, Germany), by Galke GmbH (Gittelde, Germany), and by the botanical garden of the TU Braunschweig (Braunschweig, Germany). We thank C. Kakoschke and B. Jaschok-Kentner for NMR measurements.

Table 3. ^{1}H NMR Data of Procyanidin B6 in Acetone-d_6 (400 MHz, 260 K)

ring	position	major rotamer (53%) δ multiplicity (J)	minor rotamer (47%) δ multiplicity (J)
		Extension Unit	
C	H2[a]	4.54 d (7.4)	4.43−4.36 m
C	H3[a]	4.43−4.36 m	
C	OH3	4.66	
C	H4[a]	4.43−4.36 m	
A	H6[b]	5.93 d (2.4) + 5.91 d (2.4)	
A	H8[b]	5.88 d (2.4) + 5.87 d (2.4)	
B	H6′	6.79 m	
B	H5′	6.78 m	
B	H2′	6.96 d (1.8) + 6.95 d (1.8)	
		Terminal Unit	
F	H2[c]	4.46 d (8.2)	4.48 d (8.2)
F	H3	3.97 m	3.95 m
F	OH3	4.30	
F	H4B[c]	2.56 dd (8.9, 16.0)	2.46 dd (9.0, 15.9)
F	H4A[c]	2.97 dd (5.6, 16.0)	2.88 dd (5.5, 16.0)
D	H8	6.01 s + 5.87 s	
E	H6′	6.72 dd (1.8, 8.2)	
E	H5′	6.77 d (8.1)	
E	H2′	6.87 d (1.7) + 6.86 d (1.6)	
aromatic	OH	broad signals between 8.6 and 7.5	

[a] After H/D exchange with a small amount of CD_3OD two sets of signal were assigned from the 2D J resolved spectrum: Major: H2 4.51 (ΣJ 8.3), H3 4.36 (ΣJ 16.6), H4 4.38 d (9.0); Minor: H2 4.34 (ΣJ 10.0), H3 4.45 (ΣJ 17.6), H4 4.35 d (7.5). [b] Interchangeable. [c] NOEs observed in 2D ROESY spectrum between H2 and H4B, and between H3 and H-4A of terminal unit in both rotamers confirmed the (+)-catechin unit.

Table 4. ^{1}H NMR Data of Procyanidin B8 in Acetone-d_6 (400 MHz, 240 K)

ring	position	rotamer A (∼50%) δ multiplicity (J)	rotamer B (∼50%) δ multiplicity (J)
		Extension Unit	
C	H2	4.52 d (7.3) + 4.38 d (7.2)	
C	H3	4.50−4.37 m	
C	OH3	4.72	
C	H4	4.36 d (9.1) + 4.34 d (9.7)	
A	H6	5.89 d (2.4) + 5.90 d ([a])	
A	H8	5.85 d (2.3) + 5.86 d (2.3)	
B	H6′	6.81−6.75	
B	H5′	6.81−6.75	
B	H2′	6.94 d (1.4)	6.93 d (1.4)
		Terminal Unit	
F	H2[b]	4.82 s	
F	H3	4.16 m	
F	OH3	4.15 + 4.24 d (5.7)	
F	H4B[b]	2.78 br dd (2, 17)	2.71 br dd (2, 17)
F	H4A[b]	2.88 br dd (4.5, 17.0)	2.82 br dd (4.5, 17)
D	H8	6.03 s + 5.90 s	
E	H6′	6.81−6.75	
E	H5′	6.81−6.75	
E	H2′	7.03 d (1.6)	7.04 d (1.6)
aromatic	OH	6.97 and broad signals between 9.14 and 7.60	

[a] Overlap prevented determination of magnitude of coupling. [b] NOEs observed in 2D ROESY spectrum between H2 and both H3 and H4A, and between H3 and both H-4A and H-4B of the terminal unit in both rotamers confirmed the (−)-epicatechin unit.

LITERATURE CITED

(1) Shoji, T.; Mutsuga, M.; Nakamura, T.; Kanda, T.; Akiyama, H.; Goda, Y. Isolation and structural elucidation of some procyanidins from apple by low-temperature nuclear magnetic resonance. *J. Agric. Food Chem.* **2003**, *51*, 3806–3813.

(2) Dixon, R. A.; Xie, D. Y.; Sharma, S. B. Proanthocyanidins - a final frontier in flavonoid research? *New Phytol.* **2005**, *165*, 9–28.

(3) Porter, L. J. Flavans and proanthocyanidins. In *The Flavonoids: Advances In Research Since 1980*; Harborne, J. B., Ed.; Chapman and Hall Ltd: London, 1988; pp 21−62.

(4) Czochanska, Z.; Foo, L. Y.; Newman, R. H.; Porter, L. J. Polymeric proanthocyanidins. Stereochemistry, structural units, and molecular weight. *J. Chem. Soc., Perkin Trans. 1* **1980**, *1980*, 2278–2286.

(5) Kanda, T.; Akiyama, H.; Yanagida, A.; Tanabe, M.; Goda, Y.; Toyoda, M.; Teshima, R.; Saito, Y. Inhibitory effects of apple polyphenol on induced histamine release from RBL-2H3 cells and rat mast cells. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 1284–1289.

(6) Kojima, T.; Akiyama, H.; Sasai, M.; Taniuchi, S.; Goda, Y.; Toyoda, M.; Kobayashi, Y. Anti-allergic effect of apple polyphenol on patients with atopic dermatitis: A pilot study. *Allergol. Int.* **2000**, *49*, 69–73.

(7) Akiyama, H.; Sakushima, J.; Taniuchi, S.; Kanda, T.; Yanagida, A.; Kojima, T.; Teshima, R.; Kobayashi, Y.; Goda, Y.; Toyoda, M. Antiallergic effect of apple polyphenols on the allergic model mouse. *Biol. Pharm. Bull.* **2000**, *23*, 1370–1373.

(8) Yanagida, A.; Kanda, T.; Tanabe, M.; Matsudaira, F.; Oliveira Cordeiro, J. G. Inhibitory effects of apple polyphenols and related compounds on cariogenic factors of Mutans streptococci. *J. Agric. Food Chem.* **2000**, *48*, 5666–5671.

(9) Inokuchi, J.; Okabe, H.; Yamauchi, T.; Nagamatsu, A.; Nonaka, G.; Nishioka, I. Antihypertensive substance in seeds of *Areca catechu* L. *Life Sci.* **1986**, *38*, 1375–1382.

(10) Koga, T.; Moro, K.; Nakamori, K.; Yamakoshi, J.; Hosoyama, H.; Kataoka, S.; Ariga, T. Increase of antioxidative potential of rat plasma by oral administration of proanthocyanidin-rich extract from grape seeds. *J. Agric. Food Chem.* **1999**, *47*, 1892–1897.

(11) Okuda, T.; Kimura, Y.; Yoshida, T.; Hatano, T.; Okuda, H.; Arichi, S. Studies on the activities of tannins and related compounds from medical plants and drugs. I. Inhibitory effects on lipid peroxidation in mitchondria and microsomes of liver. *Chem. Pharm. Bull.* **1983**, *31*, 1625–1631.

(12) Ariga, T.; Koshiyama, I.; Fukushima, D. Antioxidative properties of proanthocyanidins. Part I. Antioxidative properties of procyanidins B-1 and B-3 from Azuki beans in aqueous systems. *Agric. Biol. Chem.* **1988**, *52*, 2717–2722.

(13) Saint-Cricq de Gaulejac, N.; Vivas, N.; Freitas, V. de; Bourgeois, G. The influence of various phenolic compounds on scavenging activity assessed by an enzymatic method. *J. Sci. Food Agric.* **1999**, *79*, 1081–1090.

(14) Matthews, S.; Mila, I.; Scalbert, A.; Pollet, B.; Lapierre, C.; Hervé du Penhoat, C. L. M.; Rolando, C.; Donnelly, D. M. X. Method for estimation of proanthocyanidins based on their acid depolymerization in the presence of nucleophiles. *J. Agric. Food Chem.* **1997**, *45*, 1195–1201.

(15) Hellström, J. K.; Törrönen, A. R.; Mattila, P. H. Proanthocyanidins in common food products of plant origin. *J. Agric. Food Chem.* **2009**, *57*, 7899–7906.

(16) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Gebhardt, S.; Prior, R. L. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J. Nutr.* **2004**, *134*, 613–617.

(17) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R. L. Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J. Agric. Food Chem.* **2003**, *51*, 7513–7521.

(18) Arts, I. C. W.; van de Putte, B.; Hollman, P. C. H. Catechin contents of foods commonly consumed in the Netherlands. 1. Fruits, vegetables, staple foods, and processed foods. *J. Agric. Food Chem.* **2000**, *48*, 1746–1751.

(19) Arts, I. C. W.; van de Putte, B.; Hollman, P. C. H. Catechin contents of foods commonly consumed in the Netherlands. 2. Tea, wine, fruit juices, and chocolate milk. *J. Agric. Food Chem.* **2000**, *48*, 1752–1757.

(20) Esatbeyoglu, T.; Winterhalter, P. Preparation of dimeric procyanidins B1, B2, B5, and B7 from a polymeric procyanidin fraction of

black chokeberry (*Aronia melanocarpa*). *J. Agric. Food Chem.* **2010**, *58*, 5147–5153.
(21) Köhler, N.; Wray, V.; Winterhalter, P. New approach for the synthesis and isolation of dimeric procyanidins. *J. Agric. Food Chem.* **2008**, *56*, 5374–5385.
(22) Kennedy, J. A.; Jones, G. P. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001**, *49*, 1740–1746.
(23) Köhler, N.; Winterhalter, P. Large-scale isolation of flavan-3-ol phloroglucinol adducts by high-speed counter-current chromatography. *J. Chromatogr., A* **2005**, *1072*, 217–222.
(24) Peterson, J.; Dwyer, J. Taxonomic classification helps identify flavonoid-containing foods on a semiquantitative food frequency questionnaire. *J. Am. Diet. Assoc.* **1998**, *98*, 677–685.
(25) Gu, L.; Kelm, M.; Hammerstone, J. F.; Beecher, G.; Cunningham, D.; Vannozzi, S.; Prior, R. L. Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. *J. Agric. Food Chem.* **2002**, *50*, 4852–4860.
(26) Foo, L. Y.; Porter, L. J. The phytochemistry of proanthocyanidin polymers. *Phytochemistry* **1980**, *19*, 1747–54.
(27) Köhler, N.; Wray, V.; Winterhalter, P. Preparative isolation of procyanidins from grape seed extracts by high-speed countercurrent chromatography. *J. Chromatogr., A* **2008**, *1177*, 114–125.
(28) Salas, E.; Le Guernevè, C.; Fulcrand, H.; Poncet-Legrand, C.; Cheynier, V. Structure determination and colour properties of a new directly linked flavanol−anthocyanin dimer. *Tetrahedron Lett.* **2004**, *45*, 8725–8729.

Received for review March 17, 2010. Revised manuscript received May 19, 2010. Accepted May 23, 2010. We gratefully acknowledge financial support from the German Federal Ministry of Education and Research "BMBF" (grant 0313828C).