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Flavan-3-ols and procyanidins from the bark of *Salix purpurea* L.

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From a commercial aqueous ethanolic extract obtained from the bark of *Salix purpurea* L. the flavan-3-ols catechin, epicatechin, gallocatechin, catechin-3-*O*-(1-hydroxy-6-oxo-2-cyclohexene-1-carboxylic acid)-ester, the dimeric procyanidins B1, B3 and the trimeric procyanidins epicatechin-(4ß→8)-catechin-(4α→8)-catechin and epicatechin-(4ß→8)-epicatechin-(4ß→8)-catechin were isolated. Structure elucidation was performed by NMR, CD, MS, degradation and optical rotation methods. A fraction containing higher oligomeric procyanidins was investigated by ^{13}C NMR. Data indicate an average degree of oligomerization of 4 to 5 flavan-3-ol units with dihydroxylated B-rings and predominance of 2,3-*cis*-stereochemistry.

1. Introduction

The bark of *Salix* species (Salicaceae) is traditionally used as an analgesic, antipyretic and anti-inflammatory crude drug material. The European Pharmacopoeia (2005) allows several *Salix* species, including *S. purpurea* L., *S. daphnoides* Vill. and *S. fragilis* L. containing not less than 1.5% total salicin. Commercial preparations prepared from water or ethanol/water extracts of Salicis cortex are used against rheumatic diseases, fever and headache (Meier and Meier-Liebi 1998). Though there is some information about the phytochemistry of the drug material (Meier and Meier-Liebi 1998), most was foccussed on the derivatives of salicylic alcohol regarded as active constituent for a long time. There is less information about other secondary plant constituents such as the proanthocyanidins (for review see Ferreira et al. 1999) for the three single species accepted by the European Pharmacopeia, or such data were elaborated from undefined *Salix* species (Kolodziej 1990). However, for correct characterization and quality control of the drug material, the phytochemical data of the dried bark material of each distinct species should be known.

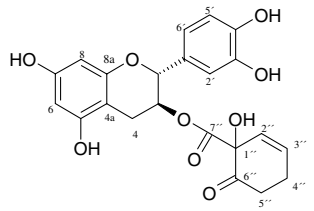
It was recently shown that the salicylic alcohol derivatives alone cannot be responsible for the overall efficacy of the crude drug (Schmidt et al. 2001) which makes it valuable to collect more knowledge about the compounds present in well defined *Salix* species which are used for crude drug collection. Because proanthocyanidins show anti-inflammatory activity *in vivo* and *in vitro* (Cos et al. 2003), this group of compounds was investigated in a commercial extract from the bark of *S. purpurea* L., one of the sources of Salicis cortex of the European Pharmacopoeia (2005), prepared by 70% ethanol. Except catechin, all compounds reported here were isolated for the first time from the bark of *S. purpurea*.

2. Investigations, results and discussion

A 70% ethanolic extract from Salicis purpureae cortex was used for the isolation of flavan-3-ols. Partition between ethyl-acetate and water gave fractions enriched with low molecular weight flavan-3-ols and oligomeric proanthocyanidins of higher m.w. respectively. Chromatography of both fractions on Sephadex® LH20 and further purification using LPLC on MCI® gel, MPLC on RP-18 and/or HPLC on RP-18 material yielded compounds **1**–**8**. Structure elucidation was performed using different spectroscopic techniques, especially NMR, MS, optical rotation and CD.

ESI-MS data, the typical NMR resonances and optical rotation data identified the monomeric flavan-3-ols as gallocatechin (**1**), catechin (**2**) and epicatechin (**3**). Their spectroscopic data were identical to those of authentic samples of our collection and published data (Baldé et al. 1991; Foo et al. 2000). The ^{1}H NMR of (**4**) in the aromatic region showed an AB-spin-system of H-6 and H-8 and an AMX-spin-system of the B-ring protons which were similar to those of catechin. The coupling constant of $^3J_{(H\text{-}2/H\text{-}3)}$ of 6.5 Hz indicates a *trans*-configuration of the heterocyclic protons H-2 and H-3. Additionally, two olefinic protons ($\delta = 5.55$ ppm and 5.97 ppm) and a multiplett counting for four aliphatic protons ($\delta = 2.24$ ppm to 2.52 ppm) were detected. The downfield shift of H-3 ($\delta = 5.24$ ppm) in comparison to catechin (δ (H-3) $= 3.99$ ppm) indicates an acyl substitution at the C-3 hydroxyl. The ESI-MS and ESI-MS-MS data suggested that a catechin-like flavan-3-ol was acylated with a cyclohexene-on-ol-carboxylic acid ($[M - H]^-$ m/z = 427.4; [M – acid moiety]$^-$ m/z = 289.3), a typical acyl moiety that occurs in polyphenols of *Salix* species. This was corroborated by the two olefinic protons ($\delta = 5.54$ and 5.97 ppm) and the 4 methylene group protons ($\delta = 2.23$ to 2.53 ppm) whose registered spectrum was in accordance with a simulated one. With its negative optical rotation compound **4** was finally identified as catechin-3-*O*-

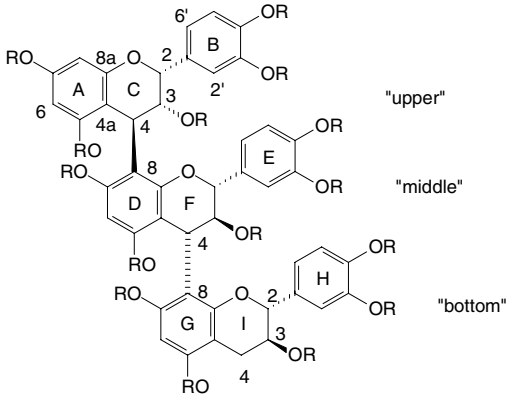
(1-hydroxy-6-oxo-2-cyclohexene-1-carboxylic acid)-ester using 2D NMR experiments (COSY, HSQC, HMBC) and by comparison with published data (Hsu et al. 1985). Comparison of the optical rotation of **4** with our own value tentatively indicates the 2,3-*trans*-configuration of catechin; however, the hitherto unknown configuration of the acyl moiety could not be established (Hsu et al. 1985). Compound **4** was first described as a constituent of the bark of *S. sieboldiana* (Hsu et al. 1985).



Catechin-3-O-(1-hydroxy-6-oxo-2-cyclohexene-1-carboxylic acid)-ester (**4**)

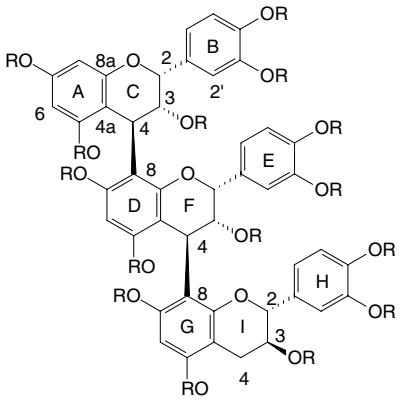
The procyanidins B1 [epicatechin-(4ß→8)-catechin] (**5**) and B3 [catechin-(4α→8)-catechin] (**6**) were the only dimeric proanthocyanidins which could be detected in the extract. After peracetylation, their structures were confirmed by different spectroscopic methods (ESI-MS, NMR, CD, optical rotation) in comparison with published values of their peracetylated derivatives (Weinges et al. 1968; Kolodziej 1986).

The molecular mass ($[M + Na]^+$ m/z = 1519) and ^{1}H NMR data of the peracetylated compound **7** (**7a**) indicated a trimeric flavan-3-ol constitution with catechol-type B-rings. Bonding positions of interflavanoid linkages were elucidated by HMBC experiments. ^{3}J cross peaks of H-4 (C and F) to the respective C-8a signals together with the connectivities of all 2D-NMR experiments clearly indicated C-4 to C-8 linkages. While the coupling constants of the "upper" unit proved a H-2/H-3 *cis*-configuration ($^3J_{(H\text{-}2/H\text{-}3)}$ = brs), the "middle" unit was *trans*-configurated ($^3J_{(H\text{-}2/H\text{-}3)}$ = 10.4 Hz). The upfield "γ-shift" of the "upper" unit of carbon C-2 (δ = 73.77 ppm) resulted from a quasiaxial orientation of the flavan-3-ol unit at C-4 (Fletcher et al. 1977). In contrast, the relative 2,3-*trans*-stereochemistry of the "bottom" unit cannot be determined with certainty by the small coupling constants of the H-2/H-3 (I) protons ($^3J_{(H\text{-}2/H\text{-}3)}$ = 3.1 Hz). However, the observed small value can be explained with conformational changes of ring I of which the catechol substituent is mainly in an axial position (Balas et al. 1995). Thus, in accordance with its optical rotation value identical to that published (Foo and Karchesy 1989), compound **7** could be identified as epicatechin-(4ß→8)-catechin-(4α→8)-catechin.



Epicatechin-(4ß→8)-catechin-(4α→8)-catechin (**7**, R = H; **7a**, R = Ac)

Compound **8** gave an $[M - H]^-$ ion peak at m/z = 865 by ESI-MS indicating a procyanidin skeleton composed of three catechin/epicatechin moieties. Cleavage according to Thompson et al. (1972) yielded catechin and procyanidin B1. The latter was identified as its peracetate by NMR and optical rotation in comparison with reference material and published data (Kolodziej 1990). Thus, the "middle" and "bottom" units could be identified as epicatechin-(4ß→8)-catechin. The ^{1}H NMR spectrum at ambient temperature of the peracetylated compound **8a** showed different sets of rotameric signals so that the complete analysis of resonances failed. Low-temperature NMR measurement at −20 °C (253 K) in the free phenolic state (**8**) gave a sufficiently resolved spectrum without or with only weak conformational rotamers. The NMR data sets were in accordance with those reported in the literature (Shoji et al. 2003) and identify compound **8** as epicatechin-(4ß→8)-epicatechin-(4ß→8)-catechin.



Epicatechin-(4ß→8)-epicatechin-(4ß→8)-catechin (**8**, R = H; **8a**, R = Ac)

Although isolation, purification and structure elucidation of oligomeric proanthocyanidins higher than tetrameric flavan-3-ols is difficult or even impossible today concerning their full stereochemistry, some fundamental information about the flavan-3-ol composition of higher oligomers can be obtained by ^{13}C NMR spectroscopy. The spectrum of an oligomeric fraction of Salicis purpureae cortex extract (Fig.) was prepared and analysed according to the literature (Newman et al. 1987; Eberhardt and Young 1994). The ratio of *cis* : *trans* isomers was estimated by integration of the signals at δ = 77 ppm and 83.5 ppm. The 5 : 1 ratio indicates that flavan-3-ols with a relative 2,3-*cis* configuration predominate in the bark of *S. purpurea*. By integration of the C-3 resonances of the extender units (δ = 73 ppm) and the resonances of C-3 of all terminal units (δ = 68 ppm), the average degree of oligomerization could be estimated to be 4 to 5 flavan-3-ol units. Notable is the absence of carbonyl- or carboxyl carbons at ca. δ = 200 ppm and 170 ppm, respectively, indicating that there is no acyl substitution in the set of oligomers. Furthermore, no evidence was found for the occurrence of proanthocyanidins with trihydroxylated B-rings by careful inspection of the expected B-ring carbon region (ca. 107–112 ppm) of prodelphinidin-type constituents.

In summary, this is the first report on the isolation and structure elucidation of monomeric and oligomeric flavan-3-ols [except catechin (Pearl and Darling 1970)] from the bark of *Salix purpurea*. Additional structure analogues with another hydroxylation pattern than 3,4-dihydroxylation in the B-ring were not observed for flavanol oligomers. It could also be shown that the higher oligomeric proanthocyanidin spectrum of *S. purpurea* consists mainly

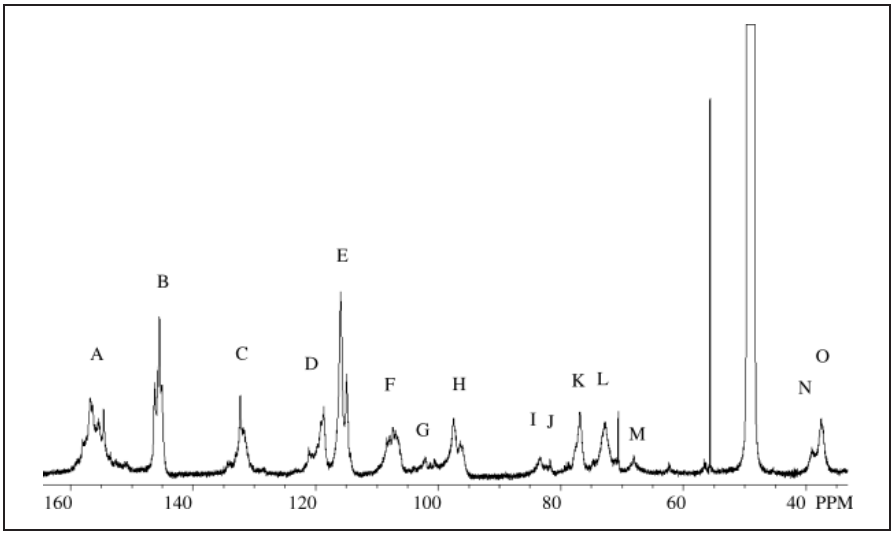


Fig.:
^{13}C NMR spectrum of an oligomeric flavan-3-ol fraction of Salicis purpureae cortex (100 MHz, MeOH-d_4). (A) C-5/C-7/C-8a of A-rings; (B) C-3′/C-4′of procyanidin units; (C) C-1′of B-rings ; (D–E) C-2′/C-5′/C-6′of PC units; (F) substituted A-ring carbons; (G) C-4a of PC units; (H) C-6/C-8 of A-rings; (I) C-2 of extender 2,3-*trans* units; (J) C-2 of terminal 2,3-*trans* units; (K) C-2 of 2,3-*cis* units; (L) C-3 of all extender units; (M) C-3 of terminal units, (N) C-4 of 2,3-*trans* units, (O) C-4 of 2,3-*cis* units

of flavan-3-ols with dihydroxylated B-rings and the predominance of 2,3-*cis* configurated units. Thus, concerning its flavanol derivates, the bark of *S. purpurea* resembles much the bark of the East-asian *S. sieboldiana* Blume that has been shown to contain compounds **2**–**8** (Hsu et al. 1985). From the bark of *S. pet-susu* Kimura **2** and **6** were isolated (Ohara and Hujimori 1996); Salicis cortex crude drug material obtained from the pharmaceutical market and not further defined taxonomically contained **2**, **3**, **5**, **6** and **8** (Kolodziej 1990).

3. Experimental

3.1. Plant material

Dry 70% ethanolic extract of Salicis pupureae cortex with a DER 8–14 : 1. The extract was obtained from Bionorica (Neumarkt/Obpf, Germany); batch number AE 0665/2 (voucher at Bionoria, D-Neumarkt; another voucher is deposited at the Institute of Pharmaceutical Biology and Phytochemistry, WWU Münster under PBMS 212). Its content of salicin derivatives was determined to 16,9% according to the European Pharmacopoeia (2005), and tannins following (Glasl 1983) to 8.5%.

3.2. Chromatographic systems

Sephadex Chromatography: Sephadex®LH20 [Pharmacia, Uppsala, Sweden]; column: 5.5 × 90 cm, flow: 0.5 mL/min; eluents: Sephadex I: 4 L EtOH/H_2O (3 + 1), followed by EtOH 2 L, EtOH/MeOH (1 + 1) 3 L and Me_2CO/H_2O (7 + 3) 3 L; Sephadex II: 4 L EtOH/H_2O (3 + 1), EtOH/MeOH (1 + 1) 1 L and Me_2CO/H_2O (7 + 3) 4 L; Sephadex III: EtOH/MeOH (1 + 1) 5 L, MeOH 7 L and Me_2CO/H_2O (7 + 3) 4 L.
LPLC on MCI: MCI®-gel [Mitsubishi Corp., Tokyo]; column: 2.5 × 100 cm, flow: 10 mL/min; linear gradient with 2 Waters Millipore® HPLC-pumps (model 510) with a Waters Millipore® Automated Gradient Controller; step gradient with one pump and 500 ml per step; MCI I: linear gradient of 20% MeOH to 70% MeOH in 400 min, then 100% MeOH; MCI II: step gradient of 30% MeOH to 50% MeOH, 5% steps, then 100% MeOH; MCI III: 10% step gradient from 20% to 70% MeOH, 500 ml per step, then 100% MeOH.
MPLC on RP-18: Orpegen HD-Sil-RP-18, 18–60 µm, column: 26 × 460 mm, pump as above; MPLC I: 10% step gradient from 20% to 80% MeOH, 500 ml per step, then 100% MeOH, flow: 10 mL/min,
Preparative HPLC: eluents: MeCN (A) and in aqua millipore® (B); flow: 6 mL; HPLC I: system: Knauer RP-18, Eurospher®100, 7 µm, 250 × 20 mm with 30% A to 60% A in 15 min; detection at 278 nm.
TLC: all fractions were tested by TLC on silica gel plats [Merck, Darmstadt, Germany]; mobile phase: EtOAc/HCO_2H/H_2O 90 + 5 + 5; detection by Naturstoffreagent (diphenylboryloxy-ethylamine), vanillin/HCl and anisaldehyde/sulphuric acid.
NMR and MS: all NMR-spectra were measured on a Varian AS 400 or Varian Unity 600 in CD_3OD; $CDCl_3$ was used for acetylated flavan-3-ols (peracetylation with Ac_2O/pyridine 1 + 1 for 24 h at room temperature); ESI-MS-data were measured on a Finnigan LCQ or a Micromass Quattro LC.

3.3. Extraction and isolation

The dry pulverized extract was dissolved in water and extracted with ethylacetate exhaustively to yield an ethylacetate phase (46%) an a water phase (54%).
The EtOAc fraction (148,4 g) was chromatographed on Sephadex 1 in four portions to give 15 fractions each run. Fraction 7 (2320 to 2780 ml, 2868 mg) was further separated by MCI I (2380 to 2580 ml, 498 mg) and MPLC I (1630 to 1970 ml) to give 382 mg of compound **4**. MCI®-separation (MCI I) of the Sephadex® fraction 9 (3140 to 3540 ml, 1347 mg) lead directly to compounds **1** (1880 to 2280 ml, 90 mg) and **2** (2530 to 3030 ml, 42 mg). Compound **3** (3130 to 3280 ml, 27 mg) was further purified by HPLC I (R_t = 10,5 min, 6 mg). Compounds **5** and **6** were separated from Sephadex® fraction 11 (4100 to 4835 ml, 1749 mg) by chromatography with MCI I (1300 to 1690 ml, 287 mg) and again with MCI II (**5**: 1160 to 1285 ml, 129 mg; **6**: 1410 to 1600 ml, 64 mg).
The water fraction (171.8 g) was chromatographed with Sephadex II to yield a sugar enriched fraction (the first 3540 ml, 94,9%) and a flavan-3-ol enriched fraction (3540 to 8840 ml, 5,1%). The flavan-3-ol-fraction again was separated with Sephadex III to give 11 fractions. Compounds **7** and **8** were purified from fraction 4 (4690 to 5490 ml, 201 mg) with MCI III (**7**: 865 to 960 ml, 23 mg; **8**: 1095 to 1775 ml, 44 mg). Fraction 11 (9000 to 10730 ml, 3342 mg) was used as the higher oligomeric fraction (Newman et al. 1987; Eberhardt and Young 1994).

3.4 Structural data

The physico-chemical data of **4**, **7a** and **8** will be presented here, because the quality of published data are worse (**4**) or not existing (**7a**) or differ (**8**) from those presented in the literature.
Catechin-3-*O*-(1-hydroxy-6-oxo-2-cyclohexene-1-carboxylic acid)-ester (**4**): $[\alpha]_{20}^{D}$ −31 (c = 0,1; acetone); ^{1}H NMR (400 MHz, MeOH-d_4, δ = 3.30 ppm, *data confirmed by simulation; δ = [ppm], [J = [Hz]]): 2.28 [dd, 2.0/5.0, H-5″a*], 2.32 [ddd, 1.8/3.8/−16.0, H-4″a*], 2.39 [dd, 1.8/3.9, H-4″b*], 2.49 [ddd, 2.0/9.0/−16.0, H-5″b*], 2.64 [dd, 6.7/−16.5, H-4a], 2.77 [dd, 5.1/−16.5, H-4b], 4.9 [d, 6.5, H-2], 5.24 [ddd, 5.1/6.1/6.7, H-3], 5.54 [ddd, 1.8/1.8/9.8, H-2″*], 5.89 [d, 2.3, H-8], 5.94 [d, 2.3, H-6], 5.97 [ddd, 3.9/3.9/9.8, H-3″*], 6.65 [dd, 2.1/8.1, H-6′], 6.73 [d, 8.1, H-5′], 6.75 [d, 2.1, H-2′]; ^{13}C-NMR (100 MHz, MeOH-d_4; *interchangeable; δ = [ppm]): 24.35 [C-4], 26.74 [C-4″], 36.17 [C-5″], 72.87 [C-3], 79.05 [C-2], 79.16 [C-1″], 95.43 [C-8], 96.49 [C-6], 99.29 [C-4a], 114.7 [C-2′], 116.3 [C-5′], 119.35 [C-6′], 128.7 [C-2″], 130.7 [C-1′], 133.22 [C-3″], 146.39 [C-4′*], 146.52 [C-3′*], 156.37 [C-8a], 157.62 [C-5], 158.26 [C-7], 170.57 [C-7″], 206.95 [C-6 ″]; ESI-MS: $[M + Na]^+$ m/z = 451,2; $[M + K]^+$ m/z = 467,2; $[M - H]^-$ m/z = 427,4; [M – acid moiety]$^-$ m/z = 289,3; ESI-MS-MS: daughters of 451,2: [M – acid moiety – $H_2O + Na]^+$ m/z = 295; [acid-moiety + $Na]^+$ m/z = 178,9; [acid moiety – $CO_2 + Na]^+$ m/z = 134,9.
Epicatechin-(4ß→8)-catechin-(4α→8)-catechin (**7**): $[\alpha]_{20}^{D}$ −193,3 (c = 0,1; MeOH); ESI-MS: $[M + Na]^+$ m/z = 1519. ^{1}H NMR of peracetate (**7a**; 400 MHz, $CDCl_3$, δ = 7.26 ppm; * interchangeable; δ = [ppm], [J = [Hz]]): 2.42 [dd, 3.9/−17.1, H-4a (I)], 2.73 [dd, 3.9/−17.1, H-4b (I)], 4.32 [d, 10.4, H-2 (F)], 4.41 [d, 2.3, H-4 (C)], 4.42 [d, 8.4, H-4 (F)], 4.91 [dd, 1.4/2.3, H-3 (C)], 5.19 [br s, H-2 (C)], 5.24 [ddd, 3.1/3.9/3.9, H-3 (I)], 5.31 [d, 3.1, H-2 (I)], 5.66 [dd, 8.4/10,4, H-3 (F)], 5.91 [d, 2.2, H-8 (A)], 6.25 [dd, 2.0/8.4, H-6′ (B)], 6.28 [d, 2.2, H-6 (A)], 6.3 [dd, 2.0/8.4, H-6′ (H)], 6.66 [s, H-6 (G)], 6.69 [s, H-6 (D)], 6.70 [d, 2.0, H-2′ (H)], 6.84 [d, 2.0, H-2′ (E)], 6.92 [dd, 2.0/8.4, H-6′ (E)], 7.05 [d, 8.4, H-5′ (B)*], 7.06 [d,

8.4, H-5′(E)*], 7.16 [d, 8.4, H-5′ (H)], 7.22 [d, 2.0, H-2′ (B)]; ^{13}C NMR of peracetate (**7a**; 100 MHz, $CDCl_3$, δ = 77.0 ppm; [a–d]assignments with the same letter are interchangeable; δ = [ppm]): 22.24 [C-4 (I)], 34.46 [C-4 (C)], 37.31 [C-4 (F)], 67.78 [C-3 (I)], 70.68 [C-3 (C)], 71.40 [C-3 (F)], 73.77 [C-2 (C)], 77.9 [C-2 (I)], 79.98 [C-2 (F)], 107.68 [C-8 (A)], 108.9 [C-6 (A)], 109.52 [C-6 (G)], 110.69 [C-4a (G)], 112.16 [C-4a (A)], 112.3 [C-6 (D)], 117.51 [C-8 (G)], 117.97 [C-4a (D)[a]], 118.56 [C-8 (D)[a]], 120.47 [C-2′ (H)], 122.89 [C-2′ (B)], 123.11 [C-2′ (E)], 123.56 [C-5′ (E)[b]], 123.48 [C-6′ (H)], 123.9 [C-5′ (B)[b]], 124.48 [C-6′ (B)], 125.33 [C-5′ (H)], 125.78 [C-6′ (E)], 135.19 [C-1′ (E)], 135.90 [C-1′ (H)], 136.64 [C-1′ (B)], 142.17 [C-3′ (B)], 142.21 [C-4′ (B)[c]], 142.58 [C-3′ (H)[c]], 142.6 [C-3′ (E)[c]], 142.66 [C-4′ (H)[c]], 142.91 [C-4′ (E)], 147.96 [C-7 (G)], 148.34 [C-5 (A)], 148.51 [C-7 (D)[d]], 148.66 [C-5 (G)[d]], 149.19 [C-5 (D)[d]], 149.88 [C-7 (A)], 151.69 [C-8a (G)], 155.75 [C-8a (D)], 156.12 [C-8a (A)].

Epicatechin-(4ß→8)-epicatechin-(4ß→8)-catechin (**8**): $[\alpha]_{20}^{D}$ +132 (c = 0,1; acetone); ESI-MS: $[M-H]^-$ m/z = 865. ^{1}H NMR (600 MHz, MeOH-d_4, δ = 3.30 ppm, 253 K, [a, b]assignments with the same letter are interchangeable; δ = [ppm], [J = [Hz]]): 2.55 [dd, 4.5/−17.1, H-4a (I)], 2.62 [dd, 5.2/−16.5, H-4b (I)], 3.99 [d, 2.0, H-3 (C)], 4.07 [brs, H-3 (F)], 4.19 [m, H-3 (I)], 4.48 [brs, H-4 (C)], 4.76 [brs, H-4 (F)], 5.02 [brs, H-2 (F)[a]], 5.03 [brs, H-2 (I)[a]], 5.07 [brs, H-2 (C)], 5.88 [s, H-6 (D)[b]], 5.89 [s, H-6 (G)[b]], 5.97 [d, 2.4, H-6 (A)], 6.01 [d, 2.4, H-8 (A)], 6.68 [d, 8.0, H-5′ (E)], 6.69 [dd, 2.3/8.0, H-6′ (B)], 6.72 [d, 8.0, H-5′ (H)], 6,73 [d, 8.0, H-5′ (B)], 6.78 [dd, 2.3/8.0 H-6′ (E)], 6.86 [d, 2.3, H-2′ (H)], 6.89 [d, 2.3, H-2′ (B)], 6.90 [dd, 2.3/8.0, H-6′ (H)], 7.04 [d, 2.3, H-2′ (E)]; ^{13}C NMR (150 MHz, MeOH-d_4, δ = 49.0 ppm; 253 K, [a–c]assignments with the same letter are interchangeable; δ = [ppm]): 26.2 [C-4 (I)], 37.0 [C-4 (C)], 37.1 [C-4(F)], 68.1 [C-3 (I)], 72.3 [C-3 (F)], 73.5 [C-3(C)], 76.8 [C-2 (C)], 76.9 [C-2 (F)], 81.6 [C-2 (I)], 95.9 [C-6 (A)[a]], 96.0 [C-8 (A)[a]], 96.8 [C-6 (D)], 97.0 [C-6 (G)], 100.5 [C-4a (G)], 101.7 [C-4a (A)], 102.5 [C-4a (D)], 106.4 [C-8 (D)], 108.1 [C-8 (G)], 113.9 [C-2′ (H)], 114.8 [C-2′ (B)[b]], 114.9 [C-2′ (E)[b]], 115.78 [C-5′ (B)[c]], 115.8 [C-5′ (E)[c]], 115.9 [C-5′ (H)[c]], 118.6 [C-6′ (E)], 119.0 [C-6′ (H)], 119.1 [C-6′ (B)], 132.4 [C-1′ (B)], 132.6 [C-1′ (H)], 132.7 [C-1′ (E)], 145.2 [C-4′ (E)], 145.3 [C-4′ (H)], 145.6 [C-4′ (B)], 145.7 [C-3′ (H)], 145.8 [C-3′ (E)], 146.0 [C-3′ (B)], 153.7 [C-8a (G)], 154.8 [C-8a (C)], 155.7 [C-5 (G)], 156.4 [C-7 (G)], 156.9 [C-7 (D)], 157.1 [C-5 (D)], 157.8 [C-5 (A)], 158.0 [C-8a (A)], 158.2 [C-7 (A)].

Cleavage reaction according to Thompson et al. (1972); in brief, 25 mg of **8** were dissolved in 4 ml 0.1 N ethanolic HCl and heated at 60 °C for 15 min. The solution was dried, again dissolved in 1.5 ml ethanol 96% and chromatographed on Sephadex®LH20 (1.5 × 7.5 cm; 5 ml fractions) with ethanol as eluent to yield **2** (2.5 mg) and **5** (3.0 mg).

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