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Prodelphinidin trimers and characterization of a proanthocyanidin oligomer from *Cistus albidus*

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Received November 3, 2002, accepted December 12, 2002

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Pharmazie 58: 416–419 (2003)

Two new natural prodelphinidin trimers have been isolated from the air-dried herb of *Cistus albidus*, epigallocatechin-(4 β $\rightarrow$ 8)-gallo catechin-(4 α $\rightarrow$ 8)-catechin and epigallocatechin-(4 β $\rightarrow$ 8)-gallo catechin-(4 α $\rightarrow$ 8)-gallo catechin in addition to catechin, gallo catechin and thirteen known proanthocyanidins. The structures were determined on the basis of 1D- and reverse 2D-NMR (HSQC, HMBC) experiments of their peracetylated derivatives, MALDI-TOF-MS and by acid-catalysed degradation with phloroglucinol. A more abundant higher oligomeric proanthocyanidins fraction was also isolated and its chemical constitution studied by ¹³C-NMR. The mean molecular weight of the higher oligomeric fraction was estimated to be 6–7 flavan-3-ol-units.

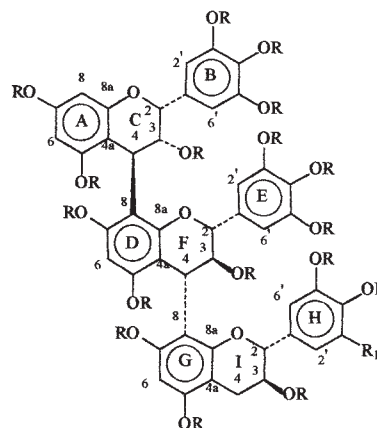
1. Introduction

Cistus albidus L. (Cistaceae), a shrub widely distributed in the Mediterranean area, is traditionally used as a tanning agent [1] and in Morocco for the treatment of gastrointestinal diseases [2]. Recently, the HPLC analysis of an ethyl-acetate soluble fraction yielded gallo catechin and catechin and indicated the presence of proanthocyanidins [3]. The knowledge of the structural variation of the proanthocyanidin fraction is of importance for a better understanding of the chemical structure of proanthocyanidins in relation to their role in tanning processes and their presumed participation on the traditional use of aqueous extracts of *Cistus* spec. as remedies for various skin diseases and as anti-inflammatory agents [4]. Moreover, aqueous extracts of *Cistus* spec. were found to have gastroprotective effects and antioxidant activity [5, 6].

2. Investigations, results and discussion

The ethyl acetate soluble fraction of an acetone/water (7:3) extract of *Cistus albidus* was fractionated by a combination of MPLC on RP-18 material, Sephadex LH-20 and MCI gel chromatography (s. Exp.). A range of flavanols and proanthocyanidins were isolated and characterized as catechin and gallo catechin, in an approximate ratio of 1:2, epicatechin-(4 β $\rightarrow$ 8)-catechin, catechin-(4 α $\rightarrow$ 8)-catechin, epigallocatechin-(4 β $\rightarrow$ 8)-catechin, gallo catechin-(4 α $\rightarrow$ 8)-catechin, epigallocatechin-(4 β $\rightarrow$ 6)-catechin, gallo catechin-(4 α $\rightarrow$ 8)-gallo catechin, gallo catechin-(4 α $\rightarrow$ 6)-catechin, epigallocatechin-(4 β $\rightarrow$ 6)-gallo catechin and gallo catechin-(4 α $\rightarrow$ 6)-gallo catechin. The identity of all flavanoids was established by physical properties (1D- and 2D NMR, circular dichroism (CD), $[\alpha]$, and MALDI-TOF-MS) of the corresponding derivatives ob-

tained after peracetylation in comparison with authentic sample from earlier work and published data [7, 8]. The remaining aqueous-phase (s. Exp.) was further fractionated on Sephadex LH-20, MCI-gel and MPLC on RP-18 material to give the compounds **1–4**.



- 1: R = H, R₁ = H
 1a: R = OAc, R₁ = H
 2: R = H, R₁ = OH
 2a: R = OAc, R₁ = OAc

Compound **1** showed a prominent quasi-molecular ion peak at m/z 1637 $M + Na^+$ in the MALDI-TOF-MS of its peracetate (**1a**), which suggests a B-type triflavanoid constitution composed of two (epi)gallo catechin units and one (epi)catechin moiety. ¹H-NMR of **1a** in CDCl₃ (600 MHz) gave two two-proton singlets at δ 6.82 and 6.88 and an AMX-spin system typical for two pyrogallol type B-ring systems and one catechol-type-B ring, respectively. From a homonuclear 2D COSY spectrum the heterocyclic proton spin systems of the C and F-rings were readily as-

signed. Subsequently, with the reverse 2D methods for heteronuclear single-bond (HSQC) and multiple-bonds (HMBC) proton-carbon connectivities were established, except for the correlation between the H-4 (I) protons and the respective C-4 (I) signal. The latter carbon signal was unambiguously determined using an APT-experiment, which showed a signal typical for the presumed $-\text{CH}_2$ -group at δ 29.76 ppm. The pyrogallol-type B- and E-ring of the extender units could be determined with the long-range couplings (4J) between the H-2 (C) and H-2 (F) proton signals and the respective H-2'/6' signals in the homonuclear 2D COSY spectrum and the heteronuclear 3J correlations between H-2 (C) and H-2 (F) through the respective C-2' and C-6' carbons. The heterocyclic coupling constants $J_{2,3(C)} < 2$ Hz and $J_{2,3(F)} = 10.2$ Hz confirmed the relative 2,3-*cis*- and 2,3-*trans* stereochemistry of the different extender units corresponding to an epigallocatechin and gallocatechin moiety, respectively. The up-field position of the C-2 (C) resonance at δ 72.68 ppm indicated the aryl group at C-4 placed in a quasiaxial orientation (" γ -effect") [9] in conjunction with the correlation between H-8 (A) and H-4 (C) to the C-8a (A) confirmed the "upper" flavan-3-ol unit as epigallocatechin. In contrast, the relative 2,3-stereochemistry of the terminal unit can not be determined with the small coupling constant of the H-2 (I) proton. The observed broad singlet at δ 5.40 ppm reveals the presence of an epicatechin rather than a catechin moiety. This observation has already been reported for the peracetylated catechin-(4 $\alpha \rightarrow$ 8)-catechin-(4 $\alpha \rightarrow$ 8)-catechin and can be explained with conformational changes of ring I of which the catechol substituent is mainly in an axial position [10]. Therefore, the structure elucidation was corroborated by acid-catalysed reaction of **1** in the presence of phloroglucinol to give epigallocatechin-(4 $\beta \rightarrow$ 2)-phloroglucinol and gallocatechin-(4 $\alpha \rightarrow$ 8)-phloroglucinol as major addition products and catechin as releasing terminal flavan-3-ol [11]. The degradation products were identified by comparison of the spectroscopic properties (NMR, $[\alpha]$, CD) of their peracetates with authentic samples, which were liberated under the condition employed by acid-degradation of the well documented prodelphinidin dimers epigallocatechin-(4 $\beta \rightarrow$ 8)-catechin and gallocatechin-(4 $\alpha \rightarrow$ 8)-gallocatechin. A detailed discussion of the spectral behavior of the phloroglucinol adducts can be found elsewhere [12]. The location of the

interflavanoid linkages was recognized for **1a** by long-range correlations (HMBC) of the H-4 (C) with the C-8a (D) and the H-4 (F) with the C-8a (G) [10]. These key correlations indicate that the flavan-3-ol units are C-4/C-8 linked.

Compound **2** showed a prominent ion peak at m/z 1694 in the MALDI-TOF-MS $M + \text{Na}^+$ of its peracetate (**2a**), indicative of a trimeric proanthocyanidin composed of three gallocatechin/epigallocatechin units. ^1H NMR of **2a** in CDCl_3 (600 MHz) gave three sharp two-proton singlets at δ 6.38, 6.81 and 7.03 ppm typical for pyrogallol-type-B rings of the constituent flavan-3-ol units. NMR behaviour of **2a** was similar to compound **1a**, including the indistinct 2,3-stereochemistry of the lower flavan-3-ol unit. The C-4/C-8 bonding position of the interflavanoid linkages was recognized as well by the ^1H - ^{13}C long-range correlations (HMBC) of the H-4 (C) with the C-8a (D) and the H-4 (F) with the C-8a (G) [10]. The broad signal around δ 73 ppm (C-2 (C)) in the ^{13}C NMR spectrum of **2a** suggested that the upper flavanoid unit at C-4 is β -orientated. Epigallocatechin-(4 $\beta \rightarrow$ 2)-phloroglucinol, gallocatechin-(4 $\alpha \rightarrow$ 2)-phloroglucinol and gallocatechin were obtained on treatment compound **2** with phloroglucinol under acid conditions and identified as above described [11]. Thus, compound **2** was identified as epigallocatechin-(4 $\beta \rightarrow$ 8)-gallocatechin-(4 $\alpha \rightarrow$ 8)-gallocatechin. Compounds **3** and **4** were isolated from the same aqueous phase and identified as gallocatechin-(4 $\alpha \rightarrow$ 8)-gallocatechin-(4 $\alpha \rightarrow$ 8)-catechin and gallocatechin-(4 $\alpha \rightarrow$ 8)-gallocatechin-(4 $\alpha \rightarrow$ 8)-gallocatechin, respectively, in comparison of the spectroscopic data of their peracetates with published values [7, 8].

The oligomer fraction (obtained s. Exp.) showed an optical rotation of $[\alpha]_{578}^{20} + 60^\circ$ (C0.1, MeOH) which corresponds to a molar proportion of subunits with 2,3-*cis* stereochemistry of 77% [13]. A ^{13}C NMR spectrum of the oligomer preparation is shown in the Fig. Assignments for the resonances observed were made using those reported in the literature for isolated polymer preparations [14–17]. Accordingly, the integration of the signals at δ 115–116 ppm and 107 ppm led to an estimation of 1:9 for the procyanidin (PC):prodelphinidin (PD) ratio 14. A ca. 3.5:1 ratio was obtained for *cis*:*trans* isomers by integration of the signals close to δ 77 ppm and δ 84 ppm 16. The mean molecular size of the oligomer was estimated to be 6–7 flavan-3-ol units by integration of the C-3 signals

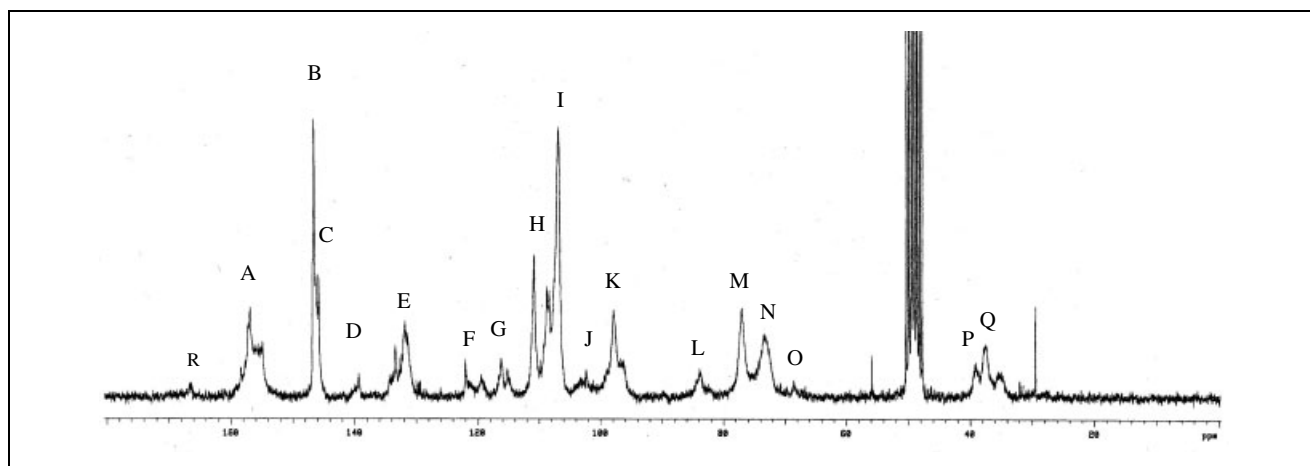


Fig. 1: Solution-state ^{13}C NMR spectrum of general features of *Cistus albidus* oligomer by 50-MHz ($\text{MeOH}-d_4$). A, C-5/C-7/C-8a of A-rings; B, C-3'/C-5' of PD units and C-3'/C-5' of galloyl units; C, C-3'/C-4' of PC units; D, C-4' of galloyl units; E, C-1' of B-rings and C-4' of PD units; F, C-1' of galloyl units; G, C-2'/C-5'/C-6' of PC units; H, C-2'/C-6' of galloyl units; I, C-2'/C-6' of PD units; J, C-4a of 2,3-*cis* units; K, C-6/C-8 of A-rings; L, C-2 of 2,3-*trans* units; M, C-2 of 2,3-*cis* units; N, C-3 of all extender units; O, C-3 of terminal flavan-3-ol units; P, C-4 of 2,3-*trans* units; Q, C-4 of 2,3-*cis* units; R, carbonyl carbons of galloyl units.

of the extender and terminal flavan-3-ol units by δ 73 ppm and near δ 68 ppm, respectively. The presence of gallate units was obvious by the carbon chemical shift at δ 110, 122 and 139 ppm as well as the carbonyl carbon chemical shift at δ 166 ppm [18].

In conclusion, besides 13 known proanthocyanidins and 2 flavanols as starter units, two new trimeric prodelfinidins (**1**, **2**) could be isolated and identified from an acetone-water extract of *Cistus albidus*. The ^{13}C NMR spectrum of the oligomeric mixture exhibited resonances typical of the predominance of subunits with 2,3-*cis*-configuration and 3',4',5'-trihydroxylated B-rings. The occurrence of galloylated oligomers and the absence of such derivatives during the low molecular polyphenols posed the question to the biogenetically regulation of the galloylation step. Future investigations deal with the pharmacological test of individual compounds to prove their presumed participation in the traditional use of *Cistus* extracts as an anti-inflammatory agent.

3. Experimental

3.1. General procedures

^1H NMR spectra were recorded in CDCl_3 (except for the oligomers, see fig.) on a Varian Gemini 200 (200 MHz) or a Bruker AM 600 (600 MHz) relative to CHCl_3 . ^{13}C NMR were recorded at 50 or 150 MHz. CD data were obtained in MeOH on a Jasco J 600. MALDI-TOF mass spectrometer: LAZARUS II (home built), N₂-laser (LSI VSL337ND) 337 nm, 3 ns puls width, focus diameter 0.1 mm, 16 kV acceleration voltage, 1 m drift length, data logging with LeCroy9450A, 2.5 ns sampling time and expected mass accuracy $\pm 0.1\%$, sample preparation: acetylated compounds were deposited from a solution in CHCl_3 on a thin layer of 2,5-dihydroxybenzoic acid (DHB) crystals. Analytical TLC was carried out on aluminium sheets (Kieselgel 60 F₂₅₄, 0.2 mm, Merck) using Me_2CO -toluene- HCO_2H (60:30:10, system A). Compounds were visualized by spraying with vanillin-HCl reagent and 1% ethanolic FeCl_3 solution. Prep. TLC was performed on silica gel plates (Kieselgel 60 F₂₅₄, 0.5 mm, Merck) using system A. Acetylation were performed in pyridine- Ac_2O (1:1.2) at ambient temp for 24 h.

3.2. Plant material

Cistus albidus L. was collected at Massiv d' Estérel/France (07/1996) and identified in comparison with authentic *Cistus albidus* obtained from the Botanical Institute, University Cologne. A voucher specimen is deposited at the Herbarium of the Institut für pharmazeutische Biologie, Münster under PBMS 188.

3.3. Extraction and isolation

Air-dried material (2 kg) was exhaustively extracted with $\text{Me}_2\text{CO}/\text{H}_2\text{O}$ (7:3, 12 l) and the combined extracts evaporated *in vacuo* to 1.5 l, filtered to remove the pptd chlorophyll, concentrated and defatted with petrol. Successive extractions with EtOAc (7.5 l) gave, on evaporation of solvent solid of 32.5 g. 17.5 of the EtOAc fraction were successively (7 $\times$ 2.5 g) applied to MPLC (Orpogen RP-18, 18–30–60 μm , 26 $\times$ 460 mm, Büchi) using (a) $\text{MeOH}-\text{H}_2\text{O}$ (7:13) 0–30 min and (b) MeOH 30–60 min at a flow rate of 20 ml min^{-1} to afford two fractions, A (10–30 min, 6.8 g) and B (30–60 min, 10.7 g; containing flavonoid glycosides to be published). Subsequent CC of fraction A (Sephadex LH-20; 55 $\times$ 900 mm; eluants: EtOH 6 l, EtOH-MeOH 1:1 8 l, Aceton- H_2O 7:3 3.5 l, 15 ml frs.) afforded the following subfractions (first 900 ml of eluant discarded).

Frs. 158–216 (1175 mg) were subjected to chromatography on MCI-gel CHP 20 P (25 $\times$ 250 mm) with a 10–50% MeOH linear gradient (17 ml/frs.) to afford catechin (subfrs. 89–112, 750 mg). Frs. 217–389 (2217 mg) were separated on MCI-gel with the same gradient as above to afford galocatechin (subfrs. 49–71, 1420 mg). Frs. 390–485 (215 mg) were separated on MCI-gel to afford subfrs. 87–115 (80 mg). A portion of the subfrs. were acetylated and purified on prep. TLC to yield the peracetates of epicatechin-(4 β $\rightarrow$ 8)-catechin and catechin-(4 α $\rightarrow$ 8)-catechin. Epigallocatechin-(4 β $\rightarrow$ 8)-catechin was achieved from frs. 486–534 (141 mg) followed by MCI-gel chromatography (subfrs. 77–97, 47 mg). Galocatechin-(4 α $\rightarrow$ 8)-catechin was isolated from frs. 535–645 (323 mg) and MCI-gel chromatography (subfrs. 65–100, 210 mg). Epigallocatechin-(4 β $\rightarrow$ 8)-catechin and galocatechin-(4 α $\rightarrow$ 8)-galocatechin were achieved from frs. 646–785 (413 mg) followed by MCI-gel chromatography as described above (subfrs. 36–58, 240 mg). Frs. 785–842 (160 mg) were subjected to

chromatography on MCI-gel elution to afford epigallocatechin-(4 β $\rightarrow$ 6)-galocatechin (subfrs. 72–90, 15 mg) and galocatechin-(4 α $\rightarrow$ 6)-catechin (subfrs. 96–111, 30 mg). Galocatechin-(4 α $\rightarrow$ 6)-galocatechin was achieved from frs. 843–899 (65 mg) and purification on MCI-gel (subfrs. 61–81, 15 mg). All compounds were identified after acetylation in comparison their physical data (NMR, MS, CD) with those of authentic samples and published values [7, 8].

The remaining H_2O -phase was evaporated to dryness (200 g). A portion (2 $\times$ 50 g) of the H_2O -phase was successively applied to CC on Sephadex LH-20 (55 $\times$ 900 mm) with 3 l EtOH- H_2O (3:1), 6.5 l EtOH- H_2O - Me_2CO (1:1:2) and 2.5 l $\text{Me}_2\text{CO}-\text{H}_2\text{O}$ (3:7) to afford 10 fractions (I-X; first 1140 ml of eluant discarded).

3.3.1. Epigallocatechin-(4 β $\rightarrow$ 8)-galocatechin-(4 α $\rightarrow$ 8)-catechin (**1**)

Fr. II (3724–4959 ml elution volume, 996 mg) obtained from Sephadex LH-20 column was subjected to chromatography on MCI-gel CHP 20P (25 $\times$ 450 mm) with a 10–50% MeOH linear gradient (17 ml/subfrs.) to afford a amorphous powder (subfrs. 15–18, 20 mg) 12: $[\alpha]_D^{20} = -176^\circ$ (C0.10, MeOH). 10 mg were acetylated to give **12a**: MALDI-TOF-MS: $[\text{M} + \text{Na}]^+ m/z$ 1637. ^1H NMR (CDCl_3 , 600 MHz): δ 1.8–2.4 (m, OAc), δ 2.28 [m, H-4 (I)], δ 2.63 [m, H-4 (I)], δ 4.28 [d, J = 10.2 Hz, H-2 (F)], δ 4.38 [d, J = 10 Hz, H-4 (F)], δ 4.39 [brs, H-4 (C)], δ 4.93 [brs, H-3 (C)], δ 5.14 [brs, H-2 (C)], δ 5.19 [m, H-3 (I)], δ 5.40 [brs, H-2 (I)], δ 5.58 [dd, J = 10 and 10.2 Hz, H-3 (F)], δ 6.05 [d, J = 2.4 Hz, H-8 (A)], δ 6.23 [dd, J = 2.0 and 8.4 Hz, H-6' (H)], δ 6.29 [d, J = 2.4 Hz, H-6 (A)], δ 6.47 [d, J = 2.0 Hz, H-2' (H)], δ 6.65 [s, H-6 (G)], δ 6.68 [s, H-6 (D)], δ 6.82 [brs, H-2'/H-6' (E)], δ 6.88 [s, H-2'/H-6' (B)], δ 7.03 [d, J = 8.4 Hz, H-5' (H)]. ^{13}C NMR (CDCl_3 , 150 MHz; * interchangeable): δ 29.76 [C-4 (I)], δ 33.78 [C-4 (C)], δ 36.33 [C-4 (F)], δ 67.04 [C-3 (I)], δ 68.73 [C-3 (C)], δ 71.18 [C-3 (F)], δ 72.68 [C-2 (C)], δ 76.46 [C-2 (I)], δ 78.97 [C-2 (F)], δ 106.98 [C-8 (A)], δ 108.28 [C-6 (A)], δ 108.58 [C-6 (G)], δ 109.97 [C-4a (G)], δ 111.41 [C-4a (A)*], δ 111.44 [C-6 (D)*], δ 116.94 [C-4a (D)], δ 117.01 [C-8 (G)], δ 117.96 [C-8 (D)], δ 119.24 [brs, C-2' (H), C-2' (E) and C-6' (E)], δ 119.36 [C-2' (B) and C-6' (B)], δ 122.56 [C-6' (H)], δ 124.25 [C-5' (H)], δ 134.26 [C-4' (B)], δ 134.37 [C-1' (E)], δ 134.58 [C-4' (E)], δ 135.03 [C-1' (H)], δ 135.19 [C-1' (B)], δ 141.61 [C-4' (H)], δ 141.92 [C-3' (H)], δ 142.89 [C-3' (E) and C-5' (E)], δ 143.13 [C-3' (B) and C-5' (B)], δ 147.06 [C-7 (G)], δ 147.50 [C-5 (A)], δ 147.79 [C-7 (D)], δ 148.04 [C-5 (G)], δ 148.36 [C-5 (D)], δ 149.19 [C-7 (A)], δ 150.69 [C-8a (G)], δ 155.05 [C-8a (D)], δ 155.45 [C-8a (A)]. Purified proanthocyanidin (10 mg) was 15 min at RT reacted with phloroglucinol (10 mg) in 1% HCl in EtOH (1 ml) with continuous shaking. The solution was then concentrated under a stream of N₂ to dryness and purified on prep. TLC in system A. The mixture of phloroglucinol adduct was further purified on prep. TLC on Cellulose (t-BuOH- $\text{CH}_3\text{COOH}-\text{H}_2\text{O}$ 60:20:20) to yield epigallocatechin-(4 β $\rightarrow$ 2)-phloroglucinol (2.2 mg), galocatechin-(4 α $\rightarrow$ 2)-phloroglucinol (2 mg) and catechin (2.3 mg).

3.3.2. Epigallocatechin-(4 β $\rightarrow$ 8)-galocatechin-(4 α $\rightarrow$ 8)-galocatechin (**2**)

Fr. III (4960–5283 ml, 813 mg) obtained from Sephadex LH-20 column was subjected to the above described MCI-gel column to afford a amorphous powder (subfrs. 18–23, 48 mg) 12: $[\alpha]_D^{20} = -173^\circ$ (C0.10, MeOH). 38 mg were acetylated to give **13a**: MALDI-TOF-MS: $[\text{M} + \text{Na}]^+ m/z$ 1694. ^1H NMR (CDCl_3 , 600 MHz): ^1H NMR (CDCl_3 , 600 MHz): δ 1.8–2.33 (m, OAc), δ 2.28 [m, H-4 (I)], δ 2.68 [m, H-4 (I)], δ 4.28 [d, J = 2.9 Hz, H-4 (C)], δ 4.29 [d, J = 10.2 Hz, H-2 (F)], δ 4.38 [d, J = 10 Hz, H-4 (F)], δ 5.13 [brs, H-3 (C)], δ 5.15 [m, H-3 (I)], δ 5.19 [brs, H-2 (C)], δ 5.39 [brs, H-2 (I)], δ 5.48 [dd, J = 10 and 10.2 Hz, H-3 (F)], δ 6.03 [d, J = 2.4 Hz, H-8 (A)], δ 6.26 [d, J = 2.4 Hz, H-6 (A)], δ 6.38 [s, H-2'/H-6' (H)], δ 6.58 [s, H-6 (D)], δ 6.65 [s, H-6 (G)], δ 6.81 [s, H-2'/H-6' (E)], δ 7.03 [s, H-2'/H-6' (B)]. ^{13}C NMR (CDCl_3 , 150 MHz; * interchangeable): δ 29.67 [C-4 (I)], δ 34.02 [C-4 (C)], δ 36.32 [C-4 (F)], δ 67.02 [C-3 (I)], δ 68.91 [C-3 (C)], δ 71.18 [C-3 (F)], δ 72.98 [C-2 (C)], δ 76.26 [C-2 (I)], δ 78.70 [C-2 (F)], δ 106.93 [C-8 (A)], δ 108.36 [C-6 (A)], δ 108.72 [C-6 (G)], δ 109.92 [C-4a (G)], δ 110.88 [C-6 (D)], δ 111.57 [C-4a (A)], δ 116.55 [C-8 (D)], δ 116.91 [C-2' (H) and C-6' (H)], δ 116.98 [C-8 (G)], δ 117.66 [C-4a (D)], δ 119.18 [C-2' (E) and C-6' (E)], δ 119.34 [C-2' (B) and C-6' (B)], δ 134.24 [C-4' (H)], δ 134.34 [C-4' (H)], δ 134.38 [C-1' (E)], δ 134.52 [C-4' (E)], δ 135.07 [C-1' (H)], δ 135.46 [C-1' (B)], δ 142.87 [C-3' (E) and C-5' (E)], δ 143.19 [C-3' (B) and C-5' (B)], δ 143.45 [C-3' (H) and C-5' (H)], δ 147.08 [C-7 (G)], δ 147.52 [C-5 (A)], δ 147.98 [C-7 (D)*], δ 148.02 [C-5 (G)*], δ 148.06 [C-5 (D)*], δ 149.17 [C-7 (A)], δ 150.59 [C-8a (G)], δ 154.63 [C-8a (D)], δ 155.42 [C-8a (A)]. Phloroglucinol degradation of **13** (5 mg) gave epigallocatechin-(4 β $\rightarrow$ 2)-phloroglucinol (1.3 mg), galocatechin-(4 α $\rightarrow$ 2)-phloroglucinol (1.1 mg) and galocatechin (1.3 mg).

3.3.3. Galocatechin-(4 α $\rightarrow$ 8)-galocatechin-(4 α $\rightarrow$ 8)-catechin (**3**)

Compound **3** was achieved from fr. IV (5284–5569 ml, 1344 mg) and MCI-gel chromatography (subfrs. 57–65). Further purification of these subfrs. using MPLC on RP-18 material under the condition described

yielded 37 mg of 14. Gallocatechin-(4 α $\rightarrow$ 8)-gallocatechin-(4 α $\rightarrow$ 8)-gallocatechin (**4**) was isolated (90 mg) from the Sephadex fraction VI (5758–6061 ml, 1850 mg) and a combination of MCI-gel chromatography and MPLC on RP-18 material.

Acknowledgements: We acknowledge gratefully the recording of CD spectra (Abt. für Molekulare Strukturforschung, GBF Braunschweig), NMR-spectra (Dr. D. Bergenthal, Inst. f. Pharmazeutische Chemie, Münster and Dr. K. Bergander Inst. f. Organische Chemie, Münster) and MALDI-TOF-MS (Dr. H. Luftmann, Institut f. Organische Chemie, Münster).

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