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Proanthocyanidins and a phloroglucinol derivative from *Rumex acetosa* L.

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ABSTRACT

From the ethyl acetate soluble fraction of an acetone–water extract of the aerial parts of *Rumex acetosa* L. (Polygonaceae), a variety of monomeric flavan-3-ols (catechin, epicatechin, epicatechin-3-O-gallate), A- and B-type procyanidins and propelargonidins (15 dimers, 7 trimers, 2 tetramers) were isolated with 5 so far unknown natural products. *Dimers*: procyanidin B1, B2, B3, B4, B5, B7, A2, epiafzelechin-(4β→8)-epicatechin, epiafzelechin-(4β→8)-epicatechin-3-*O*-gallate (new natural product), epiafzelechin-(4β→6)-epicatechin-3-*O*-gallate (new natural product), epiafzelechin-3-*O*-gallate-(4β→8)-epicatechin-3-*O*-gallate, B2-3′-*O*-gallate, B2-3,3′-di-*O*-gallate, B5-3′-*O*-gallate, and B5-3,3′-di-*O*-gallate. *Trimers*: procyanidin C1, epiafzelechin-(4β→8)-epicatechin-(4β→8)-epicatechin (new natural product), epicatechin-(4β→8)-epicatechin-(4β→8)-catechin, cinnamtannin B1, cinnamtannin B1-3-*O*-gallate (new natural product), tentatively epicatechin-(2β→7, 4β→8)-epiafzelechin-(4α→8)-epicatechin (new natural product), and epicatechin-3-*O*-gallate-(4β→8)-epicatechin-3-*O*-gallate-(4β→8)-epicatechin-3-*O*-gallate.
Tetramers: procyanidin D1 and parameritannin A1. All compounds were elucidated by ESI-MS, CD spectra, 1D- and 2D-NMR experiments as free phenols or peracetylated derivatives and, in part, after partial acid-catalysed degradation with phloroglucinol.
A more abundant proanthocyanidin polymer was also isolated, purified and its chemical composition studied by ^{13}C NMR.
In addition a so far unknown phloroglucinolglycoside (1-*O*-β-D-(2,4-dihydroxy-6-methoxyphenyl)-6-*O*-(4-hydroxy-3,5-dimethoxybenzoyl)-glucopyranoside) was isolated.

1. Introduction

Rumex acetosa L. (Polygonaceae) is a perennial plant worldwide distributed in areas with temperate climate. The aerial parts of this so called “sorrel” are used within food technology and for phytotherapeutic use. Medicinal applications are related to the tannin content of the material, leading to adstringent effects which are useful for treatment of diarrhoe and skin irritations. Modern phytotherapeutic preparations with nationally registrated drug status in Europe contain extracts of *R. acetosa* for treatment of acute and chronic infections of the upper respiratory system [1].

The aerial parts have been reported to contain flavonoids (rutin, hyperoside, quercitrin, quercetin-3-*O*-glucuronide, avicularin, vitexin, orientin, isoorientin and their acetyl derivatives) [[2] and literature cited therein], 1,8-dihydroxyanthraquinones (chrysophanol and its 8-*O*-glucoside, physcion, physcionanthrone, emodin and its 8-*O*-glucoside, emodinanthrone, aloeemodin, acetoxyaloeemodin) [3,4] oxalic acid, flavan-3-ols with catechin and epicatechin [5], phenolic acids (gallic acid, protocatechuic acid, ferrulic acid, *p*-coumaric acid) and higher amounts of polysaccharides from the rhamnogalacturonan and arabinogalactan type with immunstimulating and antiphlogistic properties [6].

Despite the fact that the aerial parts of *R. acetosa* contain substantial amounts of tannins it seems interesting that no phytochemical details are published on the respective structural features.

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2. Experimental

2.1. Plant material

Dried plant material of *R. acetosa* L. (Herba Rumicis acetosae conc., Ch-B.: 43146115) was obtained from Caesar & Loretz GmbH, Hilden, Germany. Identification was performed by microscopic investigations. A voucher specimen is retained in the documentation file of the Institute of Pharmaceutical Biology and Phytochemistry under the code *Rumex* 1.

2.2. General experimental procedures

NMR spectra of the peracetylated derivatives were recorded in $CDCl_3$ (δ 7.26 and 77.00 ppm) on a Varian Unity plus 600, a Varian INOVA 500 or a Varian m400 spectrometer. Spectra of free-phenolic compounds were recorded in MeOD (δ 3.31 and 49.05 ppm) on a Varian m400 spectrometer. Assignment of rotameric signals are marked with H_R and C_R. MS data were obtained on a Quattro LC mass spectrometer. CD spectra were measured with a Jasco J-815 CD spectrometer in MeOH. Optical rotations were measured with a Perkin-Elmer 341 digital polarimeter in MeOH. Analytic TLC was carried out on silica gel aluminium plates (0.2 mm, Merck) using ethyl acetate/water/formic acid (90:5:5) as solvent. Compounds were visualized as red coloured spots by spaying with vanillin–HCl reagent. Preparative TLC of peracetylated compounds was performed on silica gel glass plates (0.5 mm, Merck) using toluene/acetone (7:3) as solvent. Acetylation of compounds was performed in pyridine/acetic acid anhydride (1:1) at room temperature for 24 h in the dark. Acid degradation with phloroglucinol was performed according to the method described by Fletcher et al. [7].

Zone capillary electrophoresis of the carbohydrate part from **28** was performed on a PACE 50101 Beckmann Coulter CE (Palo Alto, U.S.A) with 50 mM sodium borate buffer and 4.4 M acetonitrile at pH 10.3 on a capillary with 50 μm i.d. over 77 cm. Injection 1–5 s, detection 200 nm. Enantioselective separation of (+) and (−)-catechin with 1 mg test sample in MeOH/H_2O (8:2) in a buffer with 20 mM NaH_2PO_4, 20 mM Na_2HPO_4 (pH 7.0), 20 mM γ-hydroxypropylcyclodextrin, 100 mM sodiumdodecylsulfat according to Noe and Freissmuth [8].

2.3. Extraction and isolation

The dried, cut plant material (2.5 kg) was exhaustively extracted with cold acetone/water (7:3, 15 l, Ultraturrax®). The combined extracts were evaporated *in vacuo*, filtered to remove the precipitated chlorophyll, defatted with petroleum benzene and freeze-dried to yield the crude extract (252 g). This extract was partitioned between water and EtOAc. After removal of solvent, the residues were lyophilized to yield 215 g H_2O-soluble fraction (W) and 36 g EtOAc-soluble fraction (E). 35 g of E were fractionated by column chromatography over Sephadex® LH-20 (900×55 mm) using stepwise gradient elution with increasing polarity (ethanol (18 l)-methanol (14 l)-acetone/water 7:3 (5 l)) to give 13 fractions. Fractions were monitored by TLC. Further fractionation was performed using a combination of CC on MCI-Gel® CHP-20P (75–150 μm, Mitsubishi Chemical Industries, Tokyo, Japan, 2.5×50 cm), MPLC on RP18 material (3.6×50 cm, 18–32 μm; Besta Technik, Wilhelmsfeld, Germany), MLCCC (Ito Multilayer Coil Separator Extractor, P.C. Inc. Potoay, Maryland, U.S.A., 325 ml column, 1.6 mm i.D. at 800 rpm and 1 ml/min flow rate) with EtOAc–water (1:1, upper phase) as mobile phase, FCPC (Fast Centrifugal Partition Chromatography) on a CRC Kromaton system (Kromaton Technologies, Angers, France) at 1.600 rpm, 25 ml/min flow rate with water–EtOH–hexane–EtOAc (7:2:1:8, upper phase) as mobile phase, preparative HPLC on Silica Uptishere Diol, 6 μm, 250×21.2 mm or prep. TLC.

A portion of the above Sephadex-fraction 2 (3.8 g) was purified by MLCCC and CC on MCI-Gel (20–80% MeOH linear gradient; system 1) followed by MPLC (20–80% MeOH linear gradient) to yield **28** (102 mg). A part of fraction 3 (1.7 g) was fractionated by MLCCC followed by purification on MCI-Gel (system 1) to yield **1** (36 mg) and **2**. Compound **2** was finally purificated by prep. HPLC (ACN/MeOH/water-gradient) to yield 26 mg. A portion of fraction 4 (510 mg) was fractionated using a step gradient on MCI-Gel (20–50% MeOH, 50% MeOH isocratic, 50–80% MeOH; system 2) followed by prep. TLC of a peracetylated subfraction of impure **8** to yield **8a** (30 mg).

A part of Sephadex-fraction 5 (1 g) was at first fractionated using MCI-Gel (system 1, MeOH 20%, 2 L, than MeOH 80% 2 L, cleaning with MeOH 100%500 mL) which yielded two proanthocyanidin containing subfractions (a and b). Subfraction a contained compounds **4**–**7** which were isolated as their peracetates **4a**–**7a** after preparative TLC (KG 60 F_{254}, 0.5 mm layer, mobile phase toluene:acetone (7:3 V/V) of the peracetylated subfraction a. Subfraction b was purified by MPLC (system 2, with MeOH 20%, 2 L, than MeOH 80%, 2 L, than cleaning with MeOH 100%, 500 mL) yielding pure compound **3** (204 mg) An additional slightly red spot was observed in an accompanying MPLC subfraction after spraying with vanillin/HCl reagent. Complete acetylation of this subfraction and purification with prep. TLC (KG 60 F_{254}, 0.5 mm layer, mobile phase toluene:acetone (7:3 V/V) yielded **19a** (15 mg).

Parts of Sephadex-fraction 6 (300 mg) were separated by FCPC (water–EtOH–hexane–EtOAc 7:2:1:8 (upper phase) followed by peracetylation of all subfractions yielded the peracetates of **9**, **13**, **14**, **15** and **20** (**9a**, 14 mg; **13a**, 17 mg; **14a**, 12 mg; **15a**, 14 mg; **20a**, 25 mg).

Sephadex-fraction 7 (300 mg) was fractionated by FCPC (H_2O/EtOH/hexane/EtOAc 7:2:1:8 (upper phase) to give pure **10** (32 mg), **24** (10 mg), **11** (22 mg), **23** (38 mg) and **21** (18 mg). A subfraction showed next to spots from **23** and **21** a third slightly red spot on the TLC plate. After peracetylation of that subfraction followed by preparative TLC (conditions see above) the peracetate **16a** (7 mg) was isolated. A portion of Sephadex-fraction 8 (1.8 g) was subfractionated by FCPC (H_2O/EtOH/hexane/EtOAc 7:2:1:8 (upper phase). From the resulting subfractions compounds **17** (80 mg), **12** (520 mg), **23** (560 mg) and compound **26** (15 mg) were isolated in a pure state. Sephadex-fraction 9 (400 mg) was again fractionated by the above described FCPC system to yield pure **25** (69 mg) and **27** (45 mg). 1.5 g of Sephadex-fraction 11 was fractionated by FCPC (H_2O/MeOH/EtOAc 5:2:5) to give **18** (194 mg) from subfraction 1. Compound **22** was enriched in

subfraction 3 (315 mg). A portion could be purified using prep. HPLC to yield pure **22** (25 mg).

Preparation of the polymeric fraction was achieved and defined according to the procedure described by Foo et al. [34]. The H_2O-soluble fraction (W) obtained after extraction (30 g) was fractionated by CC on Sephadex® LH-20 (900×55 mm) with MeOH–H_2O 1:1 (17 l) and MeOH (4.2 l) until the eluent was colourless; then acetone–H_2O 7:3 (5 l) was used for elution to obtain the polymeric fraction (20.3 g).

2.3.1. Epiafzelechin-(4β→8)-epicatechin (**8**)

Compound **8** was obtained as epiafzelechin-(4β→8)-epicatechin-peracetate (**8a**): $[\alpha]_D^{20} = +46{,}77°$ ($c = 0.62$); ESI-MS: $[M+Na]^+$ *m/z* 963, 5; [2 M + Na]$^+$ *m/z* 1902,5; $[\Theta]_{210}$ 128688, $[\Theta]_{240}$ 23869, ^{1}H NMR ($CDCl_3$, 400 MHz; duplication due to dynamic rotational isomerism; two sets of signals in the ratio *ca* 3:1): δ 1.59–2.38 [3H, all *s*, aliphatic and phenolic –OAc] δ2.84–3.09 [*m*, H-4a,b (F)], 4.45 [*d*, *J* = 1.8 Hz, H-4 (C)], 4.56 [*brs*, H-2 (F)], 4.64 [*d*, *J* = 1.8 Hz, H_R-4 (C)], 5.11 [*m*, H-3 (F)], 5.19 [*brs*, H-3 (C)], 5.24 [*brs*, H_R-2 (F)], 5.31 [*m*, H_R-3 (C)], 5.39 [*brs*, H_R-2 (C)], 5.52 [*m*, H_R-3 (F)], 5.59 [*brs*, H-2 (C)], 5.99 [*d*, *J* = 2.1 Hz, H-8 (A)], 6.24 [*d*, *J* = 2.1 Hz, H-6 (A)], 6.58 [*s*, H_R-6 (D)], 6.62 [*d*, *J* = 2.1 Hz, H_R-6 (A)], 6.65 [*s*, H-6 (D)], 6.77 [*d*, *J* = 2.1 Hz, H_R-8 (A)], 6.89 [*dd*, *J* = 1.8/8.0 Hz, H-6′ (E)], 7.03 [*d*, *J* = 1.8 Hz, H-2′ (E)], 7.03 [*d*, *J* = 8.0 Hz, H-5′ (E)], 7.05 [*d*, *J* = 8.6 Hz, H_R-3′/5′ (B)], 7.09 [*d*, *J* = 8.6 Hz, H-3′/5′ (B)], 7.38 [*d*, *J* = 8.6 Hz, H_R-2′/6′ (B)], 7.46 [*d*, *J* = 8.6 Hz, H-2′/6′ (B)], ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19–22 [all *s*, –CO–$\underline{C}H_3$], 26.30 [C_R-4 (F)], 26.63 [C-4 (F)], 34.15 [C-4 (C)], 34.27 [C_R-4 (C)], 66.41 [C_R-3 (F)], 66.80 [C-3 (F)], 70.83 [C_R-3 (C)], 71.19 [C-3 (C)], 73.97 [C-2 (C)], 74.77 [C_R-2 (C)], 77.17 [C-2 (F)], 77.22 [C_R-2 (F)], 107.25 [C-8 (A)], 108.19 [C_R-8 (A)], 108.60 [C-6 (A)], 108.95 [C_R-6 (A)], 109.45 [C_R-4a (A) and/or C_R-4a (D)], 110.30 [C-6 (D)], 110.88 [C_R-6 (D)], 111.62 [C-4a (A) or C-4a (F)], 111.66 [C-4a (A) or C-4a (F)], 116.81 [C-8 (D)], 117.57 [C_R-8 (D)], 121.38 [C-3′/5′ (B)], 122.47 [C-2′ (E)], 122.74 [C-5′ (E)], 125.01 [C-6′ (E)], 127.51 [C_R-2′/6′ (B)], 127.56 [C-2′/6′ (B)], 134.32 [C_R-1′ (E)], 134.51 [C-1′ (E)], 135.26 [C-1′ (B)], 135.58 [C_R-1′ (B)], 141.60 [C-3′ (E)], 141.73 [C_R-3′ (E)], 141.92 [C-4′ (E)], 142.07 [C_R-4′ (E)], 147.57 [C_R-5 or C_R-7 (D)], 147.82 [C-5 (A) or C-7 (D)], 147.92 [C-5 (A) or C-7 (D)], 148.58 [C_R-5 or C_R-7 (D)], 149.06 [C-5 (D) or C-7 (A)], 149.12 [C-5 (D) or C-7 (A)], 149.80 [C_R-5 or C_R-7 (A)], 149.92 [C_R-5 or C_R-7 (A)], 150.37 [C-4′ (B)], 151.81 [C_R-4′ (B)], 154.17 [C-8a (D)], 155.17 [C_R-8a (A)], 155.33 [C_R-8a (D)], 155.58 [C-8a (A)], 168-171 [all *s*, –$\underline{C}O$–CH_3].

2.3.2. Epiafzelechin-(4β→8)-epicatechin-3-O-gallate (**9**)

Compound **9** was obtained as epiafzelechin-(4β→8)-epicatechin-3-*O*-gallate-peracetate (**9a**): $[\alpha]_D^{20} = +38.96°$ ($c = 0.77$); ESI-MS: $[M+Na]^+$ *m/z* 1199, 5; $[\Theta]_{212}$ 49772, $[\Theta]_{245}$ -11783, $[\Theta]_{260}$ -7172, $[\Theta]_{280}$ -16251; ^{1}H NMR ($CDCl_3$, 400 MHz; duplication due to dynamic rotational isomerism; two sets of signals in the ratio *ca* 5:1): δ 1.70–2.36 [all *s*, aliphatic and phenolic –OAc], 3.04 [*m*, H-4a,b (F)], 4.43 [*d*, *J* = 2,4 Hz, H-4 (C)], 4.45 [*d*, *J* = 2,4 Hz, H_R-4 (C)], 4.76 [*brs*, H-2 (F)], 4.82 [*brs*, H_R-2 (F)], 5.25 [*m*, H-3 (C)], 5.28 [*m*, H_R-3 (F)], 5.31 [*m*, H-3 (F)], 5.35 [*m*, H_R-3 (C)], 5.59 [*brs*, H-2 (C)], 5.68 [*brs*, H_R-2 (C)], 6.12 [*d*, *J* = 2.2 Hz, H-8 (A)], 6.50 [*d*, *J* = 2.2 Hz, H-6 (A)], 6.68 [*s*, H-6 (D)], 6.89 [*dd*, *J* = 2.0/8.4 Hz, H_R-6′ (E)], 6.96 [*dd*, *J* = 2.0/8.4 Hz, H-6′ (E)], 7.03 [*s*, H_R-6 (D)], 7.06 [*d*, *J* = 8.4 Hz, H-5′ (E)], 7.09 [*d*, *J* = 8.6 Hz, H-3′/5′ (B)], 7.12 [*d*, *J* = 2.0 Hz, H_R-2′ (E)], 7.13 [*d*, *J* = 2.0 Hz, H-2′ (E)], 7.44 [*d*, *J* = 8.6 Hz, H-2′/6′ (B)], 7.58 [2H, *s*, H_R-2″/6″ (G)], 7.67 [2H, *s*, H-2″/6″ (G)], ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19–22 [–CO–$\underline{C}H_3$], 26.52 [C-4 (F)], 34.21 [C-4 (C)], 68.74 [C-3 (F)], 71.20 [C-3 (C)], 73.23 [C-2 (C)], 77.17 [C-2 (F)], 107.50 [C-8 (A)], 108.76 [C-6 (A)], 110.57 [C-6 (D)], 111.64 [C-4a (A)], 111.71 [C-4a (D)], 116.87 [C-8 (D)], 121.42 [C-3′/5′ (B)], 121.83 [C-2′ (E)], 122.12 [C-2″/6″ (G)], 123.12 [C-5′ (E)], 124.57 [C-6′ (E)], 127.27 [C-2′/6′ (B)], 127.63 [C-1″ (G)], 134.24 [C-1′ (E)], 135.22 [C-1′ (B)], 138.88 [C-4″ (G)], 141.75/141.78 [C-3′/4′ (E)], 143.44 [C-3″/5″ (G)], 147.93 [C-5 (A) and C-7 (D)], 148.97 [C-5 (D)], 149.16 [C-7 (A)], 150.44 [C-4′ (B)], 154.09 [C-8a (D)], 155.45 [C-8a (A)], 163.91 [Carboxyl-C (G)], 167–170 [–$\underline{C}O$–CH_3].

2.3.3. Epiafzelechin-3-O-gallate-(4β→8)-epicatechin-3-O-gallate (**10**)

Compound **10** (15 mg) was peracetylated for analytical investigation to epiafzelechin-3-*O*-gallate-(4β→8)-epicatechin-3-*O*-gallate-peracetate (**10a**, 19 mg): $[\alpha]_D^{20} = -37.80°$ ($c = 0.69$); ESI-MS: $[M+NH_3]^+$ *m/z* 1430, 5; $[\Theta]_{206}$ -40635; ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.83–2.37 [3H, all s, aliphatic and phenolic –OAc], 3.06 [*m*, H-4a,b (F)], 4.45 [*d*, *J* = 2.4 Hz, H-4 (C)], 4.75 [*brs*, H-2 (F)], 5.34 [*m*, H-3 (F)], 5.50 [*m*, H-3 (C)], 5.71 [*brs*, H-2 (C)], 6.16 [*d*, *J* = 2.4 Hz, H-8 (A)], 6.28 [*d*, *J* = 2.4 Hz, H-6 (A)], 6.70 [*s*, H-6 (D)], 6.97 [*dd*, *J* = 2.1/8.5 Hz, H-6′ (E)], 7.06 [*d*, *J* = 8.5 Hz, H-5′ (E)], 7.07 [*d*, *J* = 8.6 Hz, H-3′/5′ (B)], 7.16 [*d*, *J* = 2.1 Hz, H-2′ (E)], 7.45 [*d*, *J* = 8.6 Hz, H-2′/6′ (B)], 7.59 [2H, *s*, H-2″/6″ (G)], 7.70 [2H, *s*, H-2″/6″ (H)], ^{13}C NMR ($CDCl_3$, 150 MHz): δ 19–22 [–CO–$\underline{C}H_3$], 26.53 [C-4 (F)], 34.38 [C-4 (C)], 68.72 [C-3 (F)], 72.58 [C-3 (C)], 74.43 [C-2 (C)], 77.21 [C-2 (F)], 107.41 [C-8 (A)], 109.06 [C-6 (A)], 110.59 [C-6 (D)], 111.46 [C-4a (A)], 111.76 [C-4a (D)], 116.65 [C-8 (D)], 121.73 [C-3′/5′ (B)], 121.85 [C-2′ (E)], 122.13 [C-2″/6″ (H)], 122.30 [C-2″/6″ (G)], 123.13 [C-5′ (E)], 124.66 [C-6′ (E)], 127.39 [C-1″ (G)], 127.64 [C-1″ (H)], 127.85 [C-2′/6′ (B)], 134.29 [C-1′ (E)], 134.74 [C-1′ (B)], 138.89 [C-4″ (G, H)], 141.78/141.80 [C-3′/4′ (E)], 143.36 [C-3″/5″ (G)], 143.46 [C-3″/5″ (H)], 147.89–147.93 [C-5 (A) and C-7 (D)], 149.06 [C-5 (D)], 149.24 [C-7 (A)], 150.54 [C-4′ (B)], 154.12 [C-8a (D)], 155.36 [C-8a (A)], 162.90 [Carboxyl-C (G)], 163.94 [Carboxyl-C (H)], 173–182 [–$\underline{C}O$–CH_3].

2.3.4. Epiafzelechin-(4β→6)-epicatechin-3-O-gallate (**16**)

Compound **16** was obtained as epiafzelechin-(4β→6)-epicatechin-3-*O*-gallate-peracetate (**16a**): $[\alpha]_D^{20} = +18.52°$ ($c = 0.05$); ESI-MS: $[M+Na]^+$ *m/z* 1199.2; $[\ ]_{237}$ 84028; ^{1}H NMR ($CDCl_3$, 600 MHz; 16a displayed at room temperature extremely broad and overlapping aromatic and heterocyclic absorptions due to the effect of rotational isomerism): δ 1.64–2.38 [3H, all *s*, aliphatic and phenolic –OAc], 2.84–3.10 [*m*, 2×H-4 (F)], 4.30 [*m*, H-4 and H_R-4 (C)], 5.39 [*brs*, H-2 (C)], 5.18 [*m*, H-3 (C)], 5.26 [*brs*, H-2 (F)], 5.62 [*m*, H-3 (F)], 6.67 [*s*, H-8 (D)], 6.74 [*d*, *J* = 2.0 Hz, H-8 (A)], 6.81 [*s*, H_R-8 (D)], 7.05 [*d*, *J* = 8.6 Hz, H-3′/5′ (B)], 7.21 [*d*, *J* = 8.6 Hz, H-2′/6′ (B)], 7.59 [2H, *s*, H_R-2″/6″ (G)], 7.65 [2H, *s*, H-2″/6″ (G)]; other signals were not determined with certainty.

2.3.4.1. Conversion of proanthocyanidins into anthocyanidins.
2 mg of **16a** were refluxed with 5% HCl in EtOH for 1 h. The reaction mixture was subsequently chromatographed on cellulose (Cellulose F, 0.1 mm, Merck) in HCO_2H–HCl–H_2O (10:1:3) with pelargonidin as ref. substance.

*2.3.5. Epicatechin-(4β→6)-epicatechin-3-O-gallate (**17**)*

Compound **17** (20 mg) was peracetylated for analytical investigation to epicatechin-(4β→6)-epicatechin-3-*O*-gallate-peracetate (**17a**, 28 mg): $[\alpha]_D^{20} = +22.53°$ ($c = 0.71$); ESI-MS: $[M+Na]^+$ *m/z* 1257.5; $[\Theta]_{238}$ 69716; ^{1}H NMR ($CDCl_3$, 500 MHz; 17a displayed at room temperature extremely broad and overlapping aromatic and heterocyclic absorptions in a ratio *ca* 1–1.5:1 due to the effect of rotational isomerism): δ 1.84–2.37 [3H, all s, aliphatic and phenolic –OAc], 2.90 [*dd*, *J* = 4.1/17.0 Hz, H-4a (F)], 3.04 [*m*, H-4b (F)], 4.31 [*brs*, H-4 (C)] 4.36 [*brs*, H_R-4 (C)], 5.25 [*m*, H-3 (C)], 5.27 [*brs*, H-2 (F)], 5.35 [*brs*, H-2 (C)], 5.60 [*m*, H_R-3 (F)], 5.66 [*m*, H-3 (F)], 6.60 [*d*, *J* = 2.3 Hz, H-6 (A) and H_R-6 (A)], 6.69 [*s*, H-8 (D)], 6.73 [*d*, *J* = 2.3 Hz, H_R-8 (A)], 6.75 [*d*, *J* = 2.3 Hz, H-8 (A)], 6.83 [*s*, H_R-8 (D)], 7.13 [*d*, *J* = 8.5 Hz, H-5′ (E)], 7.16 [*brs*, H_R-2′ (B or E), 7.17 [*dd*, *J* = 2.0/8.5 Hz, H-6′ (E)], 7.19 [*d*, *J* = 8.5 Hz, H_R-5′ (E)], 7.21 [*d*, *J* = 8.4 Hz, H-5′ (B)], 7.29 [*dd*, *J* = 1.9/8.4 Hz, H-6′ (B)], 7.31 [*d*, *J* = 2.0 Hz, H-2′ (E)], 7.35 [*d*, *J* = 1.9 H-2′ (B)], 7.58 [2H, *s*, H_R-2″/6″ (G)], 7.64 [2H, *s*, H-2″/6″ (G)], ^{13}C NMR ($CDCl_3$, 125 MHz): δ 19–22 [–CO–$\underline{C}H_3$], 26.40 [C-4 (F)], 34.72 [C-4 (C)], 68.12 [C-3 (F)], 68.17 [C_R-3 (F)], 70.89 [C-3 (C)], 71.02 [C_R-3 (C)], 73.83 [C_R-2 (C)], 73.87 [C-2 (C)], 76.86 [C-2 (F)], 107.35 [C-8 (A)], 107.42 [C_R-8 (A)], 108.62 [C-6 (A)], 109.71 [C_R-8 (D)], δ 110.63 [C-8 (D) and C-4a (A)], δ 110.89 [C_R-4a (D)], δ 110.92 [C-4a (D)], 116.70 [C-6 (D)], 121.88 [C-2′ (B)], 121.94 [C_R-2′ (B)], 122.00 [C_R-2′ (E)], 122.08 [C-2′ (E)], 122.22 [C_R-2″/6″ (G)], 122.32 [C-2″/6″ (G)], 123.14 [C_R-5′ (E)], 123.21 [C-5′ (E)], 123.55 [C-5′ (B)], 124.29 [C-6′ (B)], 124.37 [C_R-6′ (B)], 124.57 [C_R-6′ (E)], 124.62 [C-6′ (E)], 127.68 [C-1″ (G)], 127.77 [C_R-1″ (G)], 135.26 [C-1′ (B)], 135.32 [C_R-1′ (B)], 135.97 [C_R-1′ (E)], 136.02 [C-1′ (E)], 138.99 [C_R-4″ (G)], 139.03 [C-4″ (G)], 141.97–142.32 [C-3′/4′ (B, E)], 143.53 [C_R-3″/5″ (G)], 143.58 [C-3″/5″ (G)], 149.01 [C-5 (A) or C-5 (D) and C-7 (D)], 150.26 [C-5 (A) or C-5 (D) and C-7 (A)], 154.01 [C-8a (D)], 155.17 [C-8a (A)], 163.51 [Carboxyl-C_R (G)], 163.65 [Carboxyl-C (G)], 167–171 [–$\underline{C}$O–CH_3].

*2.3.6. Epiafzelechin-(4β→8)-epicatechin-(4β→8)-epicatechin (**19**)*

Compound **19** was obtained as epiafzelechin-(4β→8)-epicatechin-(4β→8)-epicatechin-peracetate (**19a**): $[\alpha]_D^{20} = +65.57°$ ($c = 0.61$); ESI-MS: $[M+Na]^+$ *m/z* 1461.5; $[\Theta]_{210}$ 213439, $[\Theta]_{220}$ 167395, ^{1}H NMR ($CDCl_3$, 600 MHz; duplication due to dynamic rotational isomerism; two sets of signals in the ratio *ca* 2–3:1): δ 1.4–2.38 [3H, all *s*, aliphatic and phenolic –OAc], 2.91 [*dd*, *J* = n.d./17.7 Hz, H_R-4a (I)], 2.96 [*dd*, *J* = n.d./17.7 Hz, H-4a (I)], 3.03 [*dd*, *J* = 4.5/17.7 Hz, H_R-4b (I)], 3.08 [*dd*, *J* = 4.5/17.7 Hz, H-4b (I)], 4.66/4.69 [*brs*, H_R-4 (C, F)], 4.70 [*brs*, H-4 (F)], 4.77 [*brs*, H-4 (C)], 4.98 [*m*, H_R-3 (C)], 5.11 [*brs*, H_R-2 (I)], 5.12 [*m*, H_R-3 (I)], 5.21 [*brs*, H-2 (I)], 5.35 [*m*, H-3 (C)], 5.37 [*brs*, H-2 (C)], 5.39 [*brs*, H-2 and H-3 (F)], 5.41 [*brs*, H_R-3 (I)], δ 5.47 [*m*, H-3 (I)], 5.72 [*brs*, H_R-2 (C)], 5.94 [*brs*, H_R-6 or 8 (A)], 6.26 [*brs*, H_R-8 or 6 (A)], 6.61 [*s*, H_R-6 (G)], 6.65 [*s*, H-6 (G)], 6.65 [*d*, *J* = 2.2 Hz, H-6 (A)], 6.71 [*s*, H-6 (D)], 6.76 [*d*, *J* = 2.2 Hz, H-8 (A)], 7.04 [*d*, *J* = 8.7 Hz, H-3′/5′ (B)], 7.07 [*d*, *J* = 8.5 Hz, H-5′ (E)], 7.08 [*d*, *J* = 8.7 Hz, H_R-3′/5′ (B)], 7.12 [*dd*, *J* = 1.9/8.5 Hz, H-6′ (E)], 7.17 [*d*, *J* = 1.9 Hz, H-2′ (E)], 7.17 [*d*, *J* = 8.2 Hz, H-5′ (H)], 7.19 [*dd*, *J* = 1.9/8.2 Hz, H-6′ (H)], 7.27 [*d*, *J* = 8.7 Hz, H-2′/6′ (B)], 7.29 [*d*, *J* = 1.9 Hz, H-2′ (H)], 7.47 [*d*, *J* = 8.7 Hz, H_R-2′/6′ (B)], ^{13}C NMR ($CDCl_3$, 150 MHz): δ 19–23 [–CO–$\underline{C}H_3$], 26.41 [C-4 (I)], 34.47 [C-4 (C)], 35.07 [C-4 (F)], 66.57 [C-3 (I)], 70.74 [C-3 (C)], 71.31 [C-4 (F)], 74.94 [C-2 (F)], 75.10 [C-2 (C)], 77.03 [C-2 (I)], 108.17 [C-8 (A)], 109.25 [C-6 (A)], 109.97 [C-4a (G)], 110.64 [C-6 (G)], 110.97 [C-6 (D)], 111.68 [C-4a (A)], 112.16 [C-4a (D)], 117.58 [C-8 (G)], 117.82 [C-8 (D)], 121.28 [C-2′ (E)], 121.32 [C_R-3′/5′ (B)], 121.43 [C-3′/5′ (B)], 121.66 [C-2′ (H)], 123.07 [C-5′ (E)], 123.24 [C-5′ (H)], 123.96 [C-6′ (E)], 124.09 [C-6′ (H)], 127.46 [C-2′/6′ (B)], 128.11 [C_R-2′/6′ (B)], 134.23 [C-1′ (B)], 135.20 [C-1′ (E)], 135.74 [C-1′ (H)], 141.67–142.14 [C-3′/4′ (E, H)], 147.20 [C-7 (G)], 147.61 [C-7 (D)], 148.51 [C-5 (G)], 148.58 [C-5 (D)], 149.90 [C-5/7 (A)], 150.59 [C-4′ (B)], 151.76/151.89 [C-8a (D, G)], 155.10 [C-8a (A)], 168–172 [–$\underline{C}$O–CH_3].

*2.3.7. Epicatechin-(4β→8)-epicatechin-(4β→8)-catechin (**21**)*

Compound **21** (12 mg) was peracetylated for analytical investigation to epicatechin-(4β→8)-epicatechin-(4β→8)-catechin-peracetate (**21a**, 15 mg): $[\alpha]_D^{20} = +113.04°$ ($c = 1.84$); ESI-MS: $[M+Na]^+$ *m/z* 1519.5; $[\Theta]_{212}$ 220364, $[\Theta]_{280}$ 13786, ^{1}H NMR ($CDCl_3$, 500 MHz; duplication due to dynamic rotational isomerism; two sets of signals in the ratio *ca* 2:1; signal set of the minor rotamer was not determined): δ 1.43–2.38 [3H, all *s*, aliphatic and phenolic –OAc], 2.74 [*dd*, *J* = 8.3/16.7 Hz, H-4a (I)], 3.12 [*dd*, *J* = 5.6/16.7 Hz, H-4b (I)], 4.63 [*brs*, H-4 (F)], 4.74 [*brs*, H-4 (C)], 5.07 [*d*, *J* = 7.8 Hz, H-2 (I)], 5.16 [*m*, H-3 (I)], 5.34 [*m*, H-3 (C) and H-2 (F)], 5.36 [*brs*, H-2 (C)], 5.41 [*m*, H-3 (F)], 6.64 [*d*, *J* = 2.3 Hz, H-6 (A)], 6.69 [*s*, H-6 (G)], 6.75 [*s*, H-6 (D)], 6.75 [*d*, *J* = 2.3 Hz, H-8 (A)], 6.91–7.35 [protons of the rings B, E and H]; ^{13}C NMR ($CDCl_3$, 125 MHz): δ 19–23 [–CO–$\underline{C}H_3$], 25.44 [C_R-4 (I)], 25.61 [C-4 (I)], 34.43 [C-4 (C)], 35.12 [C-4 (F)], 61.51 [C_R-3 (I)], 68.33 [C-3 (I)], 70.54 [C-3 (C)], 71.01 [C-4 (F)], 74.67 [C-2 (C)], 74.95 [C-2 (F)], 78.09 [C_R-2 (I)], 78.19 [C-2 (I)], 108.13 [C-6 (A)], 109.27 [C-8 (A)], 110.66 [C-6 (G)], 111.08 [C-6 (D)], 111.14 [C-4a (G)], 111.61 [C-4a (D)], 111.74 [C-4a (A)], 117.45/117.51 [C-8 (D, G)], 120.31–125.43 [C-2′/5′/6′ (B, E, H)], 133.31–136.90 [C-1′ (B, E, H)], 141.47–142.43 [C-3′/4′ (B, E, H)], 147.48 [C-7 (D, G)], 148.10/148.43 [C-5 (D, G)], 149.89 [C-5/7 (A)], 151.68/151.78 [C-8a (D, G)], 154.90 [C-8a (A)], 168–172 [–$\underline{C}$O–CH_3].

*2.3.8. Epicatechin-(2β→7, 4β→8)-epicatechin-(4β→8)-epicatechin (**23**)*

Compound **23** (cinnamtannin B1, 40 mg) was peracetylated for analytical investigation to Epicatechin-(2β→7, 4β→8)-epicatechin-(4β→8)-epicatechin-peracetate (**23a**, 54 mg): $[\alpha]_D^{20} = +32.7°$ ($c = 0.06$); ESI-MS: $[M+H]^+$ *m/z* 865.1; $[\Theta]_{232}$ 62021, $[\Theta]_{250}$ 5724, $[\Theta]_{258}$ -10228, $[\Theta]_{270}$ 38159, $[\Theta]_{270}$, $[\Theta]_{284}$; ^{1}H NMR ($CDCl_3$, 600 MHz, duplication due to dynamic rotational isomerism; two sets of signals in the ratio *ca* 1:1): δ 1.46–2.32 [3H, all *s*, aliphatic and phenolic –OAc], δ 2.90–3.07 [m, 2 × H-4 and 2 × H_R-4 (I)], 4.30 [*d*, *J* = 4.1 Hz, H-4 (C)], 4.30 [*d*, *J* = 2.4 Hz, H-4 (F)], 4.62 [*d*, H_R-4 (C)], 4.63 [*d*, H_R-4 (F)], 4.79 [*brs*, H-2 (I)], 5.00 [*d*, *J* = 4.1 Hz, H-3 (C)], 5.01 [*d*, *J* = 4.1 Hz, H_R-3 (C)], 5.20 [*m*, H-3 (I), H_R-2 (I) and H_R-3 (F)], 5.36 [*dd*, *J* = 2.4 and 2.6 Hz, H-3 (F)], 5.42 [*brs*, H_R-2 (F)], 5.53 [*m*, H_R-3 (I)], 5.42

[*d*, *J* = 2.6 Hz, H-2 (F)], 6.25 [*s*, H-6 (D)], 6.43 [*d*, *J* = 2.2 Hz, H-6 (A)], 6.52 [*d*, *J* = 2.2 Hz, H_R-6 (A)], 6.59 [*s*, H_R-6 (D)], 6.59 [*s*, H_R-6 (G)], 6.62 [*s*, H-6 (G)], 6.72 [*d*, *J* = 2.2 Hz, H-8 (A)], 6.85 [*d*, *J* = 2,2 Hz, H_R-8 (A)], 7.04 [*dd*, *J* = 2.0 and 8.5 Hz, H-6′ (E)], 7.05 [*dd*, *J* = 2.0 and 8.5 Hz, H-6′ (H)], 7.08 [*d*, *J* = 8.5 Hz, H-5′ (E)], 7.13–7.14 [*m*, H_R-5′ (E), H_R-5′ (H) and H_R-6′ (H)], 7.19 [*d*, *J* = 2.0 Hz, H-2′ (E)], 7.21 [*d*, *J* = 2.0 Hz, H-2′ (H)], 7.23 [*m*, H_R-2′ (H) and H_R-5′ (B)], 7.26 [*d*, *J* = 2.0 Hz, H_R-2′ (E)], 7.27 [*d*, *J* = 8.5 Hz, H_R-5′ (B)], 7.28 [*d*, *J* = 8.5 Hz, H-5′ (H)], 7.39 [*d*, *J* = 2.1 Hz, H-2′ (B)], 7.47 [*d*, *J* = 2.1 Hz, H_R-2′ (B)], 7.50 [*dd*, *J* = 2.1 and 8.6 Hz, H-6′ (B)], 7.57 [*dd*, *J* = 2.1 and 8.6 Hz, H_R-6′ (B)]; ^{13}C NMR ($CDCl_3$, 150 MHz): δ 19.4–21.2 [–CO–$\underline{C}H_3$], 26.15 [C-4 (I)], 26.30 [C_R-4 (I)], 27.38 [C-4 (C) and C_R-4 (C)], 33.44 [C-4 (F)], 33.61 [C_R-4 (F)], 66.34 [C-3 (I)], 66.70 [C-3 (C)], 67.90 [C_R-3 (C)], 69.73 [C_R-3 (F)], 70.47 [C-3 (F)], 75.33 [C-2 (F)] 75.50 [C_R-2 (F)], 76.69 [C-2 (I)], 97.78 [C_R-2(C)], 98.26 [C-2(C)], 104.17 [C_R-6 (D)], 104.77 [C-6 (D)], 106.65 [C-8 (A)], 107.10 [C_R-8 (A)], 107.29 [C_R-4a (D)], 108.16 [C_R-8 (D)], 108.44 [C-8 (D)], 108.73 [C_R-4a (D)], 109.70 [C-6 (A)], 109.77 [C_R-6 (A)], 109.92 [C_R-4a (G)], 110.26 [C-6 (G)], 110.83 [C_R-6 (G)], 110.95 [C-4a (G)], 113.05 [C_R-4a (A)], 114.18 [C-4a (A)], 116.67 [C-8 (G)], 117.80 [C_R-8 (G)], 121.36 [CR-2′ (H)], 121.73 [C-2′ (H)], 122.08 [C_R-5′ (E)], 122.80 [C-2′ (B)], 122.82 [C-5′ (B)], 122.99 [C_R-2′ (B) and C-5′ (E)], 123.03 [C_R-5′ (B)], 123.21 [C-5′ (H)], 123.31 [C_R-5′ (H)], 123.59 [C_R-6′ (H)], 123.67 [C-2′ (E)], 124.27 [C-6′ (H)], 124.34 [C_R-2′ (E)], 125.26 [C_R-6′ (B)], 125.50 [C-6′ (E)], 125.53 [C-6′ (B)], 125.94 [C_R-6′ (E)], 134.77 [C_R-1′ (E)], 134.82 [C-1′ (H)], 135.29 [C_R-1′ (B)], 135.36 [C-1′ (E)], 135.39 [C-1′ (B)], 135.46 [C_R-1′ (H)], 141.45–143.02 [C-3′/4′ and C_R-3′/4′ (B, E, H)], 148.55 [C-7 (G)], 148.58 [C_R-7 (G)], 148.10 and 148.13 [C-5/7 (D) or C-5 (A)], 148.55 [C-5 (G)], 148.58 [C_R-5 (G)], 148.77 [C_R-5 (A)], 149.45 [C-7 (A)], 149.61 and 144.66 [C-5/7 (D)], 150.17 or 150.20 [C-5/7 or C_R-7 (A)], 151.88 and 151.92 [C_R-8a (G) or C-8a (D)], 152.20 [C_R-8a (D)], 153.36 [C-8a (G)], 153.85 [C_R-8a (A)], 154.09 [C-8a (A)], 167.6–170.51 [–$\underline{C}O$–CH_3].

Degradation of 20 mg **23** with 30 mg phloroglucinol in 2 ml 1% ethanolic HCl yielded epicatechin (**2**) and 29, which were purified using a Sephadex® LH-20 column (25 × 80 mm) with first 300 ml EtOH, then 300 ml MeOH. Compound **29** (12 mg) was peracetylated for analytical investigation to epicatechin-(2β→7, 4β→8)-epicatechin-(4β→8)-phloroglucinol-peracetate (**29a**, 14 mg: $[\alpha]_D^{20}$ = + 106.82° (*c* = 0.44); ESI-MS: $[M + Na]^+$ *m/z* 1127.5.; $[\Theta]_{210}$ -34546, $[\Theta]_{230}$ 67077, $[\Theta]_{250}$ 5536, $[\ ]_{270}$ 22775, $[\Theta]_{284}$ -3387; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.56–2.33 [3H, all *s*, aliphatic and phenolic –OAc], 4.42 [*d*, *J* = 3.2 Hz, H-4 (F)], 4.60 [*d*, *J* = 4.2 Hz, H-4 (C)], 5.01 [*d*, *J* = 4.2 Hz, H-3 (C)], 5.02 [*dd*, *J* = 1.6 and 3.2 Hz, H-3 (F)], 5.52 [*d*, *J* = 1.6 Hz, H-2 (F)], 6.50 [*d*, *J* = 2.4 Hz, H-6 (A)], 6.55 [*s*, H-6 (D)], 6.84 [*d*, *J* = 2.4 Hz, H-4/6 (G)], 6.85 [*d*, *J* = 2.4 Hz, H-8 (A)], 6.94 [*d*, *J* = 2.4 Hz, H-6/4 (G)], 7.13 [*d*, *J* = 8.2 Hz, H-5′ (E)], 7.20 [*dd*, *J* = 2.0 and 8.2 Hz, H-6′ (E)], 7.26 [*d*, *J* = 2.0 Hz, H-2′ (E)], 7.27 [*d*, *J* = 8.2 Hz, H-5′ (B)], 7.48 [*d*, *J* = 2.0 Hz, H-2′ (B)], 7.58 [*dd*, *J* = 2.0 and 8.2 Hz, H-6′ (B)]; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19–21 [–CO–$\underline{C}H_3$], 27.29 [C-4 (C)], 33.82 [C-4 (F)], 67.76 [C-3 (C)], 70.14 [C-3 (F)], 75.22 [C-2 (F)], 97.75 [C-2 (C)], 104.15 [C-6 (D)], 106.71 [C-4a (F)], 107.20 [C-8 (A)], 109.70 [C-6 (A)], 113.06 [C-4a (A)], 114,39 [C-4/6 (G)], 115.24 [C-6/4 (G)], 118.59 [C-8 (D)], 120.24 [C-2 (G)], 122.82 [C-5′ (E)], 123.02 [C-2′ (B and E)], 124.74 [C-5′ (B)], 125.30 [C-6′ (B)], 126.15 [C-6′ (E)], 134.70 [C-1′ (E)], 135.23 [C-1′ (B)], 141.64–143.02 [C-3′/4′ (B and E)], 148.69–150.41 [C-5/7 (A and D); C-1/3/5 (G)], 152.04 [C-8a (D)], 155.57 [C-8a (A)], 168–172 [–$\underline{C}O$–CH_3].

*2.3.9. Epicatechin-(2β→7, 4β→8)-epiafzelechin-(4α→8)-epicatechin (**24**)*

Compound **24** (10 mg) was peracetylated for analytical investigation to epicatechin-(2β→7, 4β→8)-epiafzelechin-(4α→8)-epicatechin-peracetate (**24a**, 18 mg): $[\alpha]_D^{20}$ = + 10.36° (*c* = 1.93); ESI-MS: $[M + NH_3]^+$ *m/z* 1412.4 $[M + Na]^+$ *m/z* 1417.6; $[\Theta]_{210}$ -52052, $[\Theta]_{232}$ 25372, $[\Theta]_{250}$ 1049, $[\Theta]_{270}$ 14171, $[\Theta]_{280}$ -7602; ^{1}H NMR ($CDCl_3$, 600 MHz, duplication due to dynamic rotational isomerism; two sets of signals in the ratio *ca* 1:1): δ 1.4–2.36 [3H, all *s*, aliphatic and phenolic –OAc], 2.90–3.06 [*m*, H-4a,b and H_R-4a,b (I)], 4.29 [*d*, *J* = 4.8 Hz, H-4 (F)], 4.35 [*d*, *J* = 4.1 Hz, H-4 (C)], 4.62 [*d*, *J* = 4.8 Hz, H_R-4 (F)], 4.63 [*d*, *J* = 4.1 Hz, H_R-4 (C)], 4.80 [*brs*, H-2 (I)], 5.00 [*d*, *J* = 4.1 Hz, H-3 (C)], 5.01 [*d*, *J* = 4.1 Hz, H_R-3 (C)], 5.18 [*brs*, H_R-2 (I)], 5.18 [*m*, H_R-3 (F)], 5.21 [*m*, H-3 (I)], 5.39 [*m*, H-3 (F) and H_R-2 (F)], 5.51 [*m*, H_R-3 (I)], 5.71 [*d*, *J* = 2.3 Hz, H-2 (F)], 6.24 [*s*, H-6 (D)], 6.45 [*d*, *J* = 2.2 Hz, H-6 (A)], 6.55 [*d*, *J* = 2.2 Hz, H_R-6 (A)], 6.59 [*s*, H_R-6 (G)], 6.60 [*s*, H_R-6 (D)], 6.62 [*s*, H-6 (G)], 6.73 [*d*, *J* = 2.2 Hz, H-8 (A)], 6.86 [*d*, *J* = 2.2 Hz, H_R-8 (A)], 7.01 [*d*, *J* = 8.5 Hz, H_R-3′/5′ (E)], 7.04 [*d*, *J* = 8.5 Hz, H-3′/5′ (E)], 7.05 [*dd*, *J* = 1.9 and 8.4 Hz, H-6′ (H)], 7.21 [*d*, *J* = 1.9 Hz, H-2′ (H)], 7.23 [*d*, *J* = 1.9 Hz, H_R-2′ (H)], 7.23 [*d*, *J* = 8.6 Hz, H-5′ (B)], 7.25 [*d*, *J* = 8.5 Hz, H_R-2′/6′ (E)], 7.27 [*d*, *J* = 8.6 Hz, H_R-5′ (B)], 7.29 [*d*, *J* = 8.4 Hz, H-5′ (H)], 7.40 [*d*, *J* = 8.5 Hz, H-2′/6′ (E)], 7.41 [*d*, *J* = 2.1 Hz, H-2′ (B)], 7.48 [*d*, *J* = 2.1 Hz, H_R-2′ (B)], 7.51 [*dd*, *J* = 2.1 and 8.6 Hz, H-6′ (B)], 7.57 [*dd*, *J* = 2.1 and 8.6 Hz, H_R-6′ (B)], ^{13}C NMR ($CDCl_3$, 150 MHz): δ 19–22 [–CO$\underline{C}H_3$], 26.21 [C-4 (I)], δ 26.31 [C_R-4 (I)], 27.36 [C-4 (C)], 27.39 [C_R-4 (C)], 33.56 [C-4 (F)], 33.85 [C_R-4 (F)], 66.34 [C-3 (I)], 66.40 [C_R-3 (I)], 66.73 [C-3 (C)], 67.84 [C_R-3 (C)], 70.18 [C_R-3 (F)], 70.70 [C-3 (F)], 75.67 [C-2 (F)], 75.91 [C_R-2 (F)], 76.69 [C-3 (I)], 97.68 [C_R-2 (C)], 98.21 [C-2 (C)], 104.12 [C-6 (D)], 104.71 [C_R-6 (D)], 106.80 [C-8 (A)], 107.32 [C_R-8 (A)], 107.50 [C_R-4a (D)], 108.30 [C-8 (D)], 108.45 [C_R-8 (D)], 108.84 [C-4a (D)], 109.65 [C-6 (A)], 109.78 [C_R-6 (A)], 109.88 [C-4a (G)], 110.20 [C-6 (G)], 110.83 [C_R-6 (G)], 110.86 [C_R-4a (G)], 113.10 [C_R-4a (A)], 114.17 [C-4a (A)], 116.74 [C-8 (G)], 117.91 [C_R-8 (G)], 121.14 [C_R-3′/5′ (E)], 121.28 [C-3′/5′ (E)], 121.35 [C_R-2′ (H)], 121.66 [C-2′ (H)], 122.82 [C-2′ (B)], 122.99 [C_R-2′ (B)], 123.03 [C-5′ (B)], 123.21 [C-5′ (H)], 123.29 [C_R-5′ (H)], 124.23 [C-6′ (H)], 125.25 [C_R-6′ (B)], 125.54 [C-6′ (B)], 128.47 [C_R-2′/6′ (E)], 128.85 [C-2′/6′ (E)], 133.67 [C-1′ (E)], 134.28 [C_R-1′ (E)], 134.784 [C-1′ (H)], 135.33 [C-1′ (B)], 135.41 [C_R-1′ (B)], 135.52 [C_R-1′ (H)], 141.45–143.23 [C-3′/4′ (B, H)], 147.58 [C-7 (G)], 147.76 [C_R-7 (G)], 148.09 [C_R-5 (D)], 148.25 [C-5 (D)], 148.51 [C_R-5 (G)], 148.55 [C-5 (G)], 149.58 [C-7 (D)], 150.20 [C-5/7 (A)], 150.78 [C_R-4′ (E)], 150.99 [C-1′ (E)], 151.87 [C-8a (G)], 152.11 [C_R-8a (D)], 152.40 [C-8a (D)], 153.37 [C_R-8a (G)], 153.83 [C_R-8a (A)], 154.12 [C-8a (A)], 168–172 [–$\underline{C}O$–CH_3].

*2.3.10. Epicatechin-3-O-gallate-(2β→7, 4β→8)-epicatechin-(4β→8)-epicatechin (**25**)*

Compound **25** (20 mg) was peracetylated for analytical investigation to epicatechin-3-*O*-gallate-(2β→7, 4β→8)-

epicatechin-(4β→8)-epicatechin-peracetate (**25a**, 27 mg): $[\alpha]_D^{20} = +54.84°$ ($c = 0.93$); ESI-MS: $[M + NH_3]^+$ *m/z* 1706.5 $[M + Na]^+$ *m/z* 1711.3; $[\Theta]_{212}$ 126263, $[\Theta]_{224}$ 76799, $[\Theta]_{230}$ 83144, $[\Theta]_{253}$ 2325, $[\Theta]_{271}$ 32230, $[\Theta]_{286}$ -7304, ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.56–2.33 [3H, all *s*, aliphatic and phenolic –OAc], 2.85–2.97 [*m*, H-4a,b (I)], 4.22 [*d*, *J* = 4.3 Hz, H-4 (C)], 4.35 [*d*, *J* = 2.8 Hz, H-4 (F)], 4.74 [*s*, H-2 (I)], 5.08 [*m*, H-3 (I)], 5.12 [*d*, *J* = 4.3 Hz, H-2 (C)], 5.21 [*m*, H-3 (F)], 5.77 [*s*, H-2 (F)], 6.32 [*s*, H-6 (D)], 6.57 [*d*, *J* = 2.2 Hz, H-6 (A)], 6.63 [*s*, H-6 (G)], 6.71 [*d*, *J* = 2.2 Hz, H-8 (A)], 7.06 [*dd*, *J* = 2.0 and 8.5 Hz, H-6′ (H)], 7.12 [*d*, *J* = 8.5 Hz, H-5′ (H)], 7.12 [*d*, *J* = 8.3 Hz, H-6 (D)], 7.14 [*d*, *J* = 8.6 Hz, H-5′ (B)], 7.19 [*dd*, *J* = 2.0 and 8.3 Hz, H-6′ (E)], 7.21 [*s*, H-2″/6″ (J)], 7.31 [*d*, *J* = 2.0 Hz, H-2′ (E)], 7.36 [*d*, *J* = 2.2 Hz, H-2′ (B)], 7.36 [*d*, *J* = 1.9 Hz, H-2′ (H)], 7.43 [*dd*, *J* = 2.2 and 8.6 Hz, H-6′ (B)], ^{13}C NMR ($CDCl_3$, 125 MHz): δ 19–21 [–CO–$\underline{C}H_3$], 26.06 [C-4 (I)], 27.26 [C-4 (C)], 34.02 [C-4 (F)], 66.41 [C-3 (I)], 68.19 [C-3 (C)], 70.92 [C-3 (F)], 76.69 [C-2 (I)], 76.87 [C-2 (F)], 98.27 [C-2 (C)], 104.76 [C-6 (D)], 106.32 [C-8 (A)], 108.17 [C-8 (D)], 108.65 [C-4a (D)], 110.35 [C-6 (A)], 110.35 [C-6 (G)], 111.04 [C-4a (G)], 114.06 [C-4a (A)], 117.03 [C-8 (G)], 122.40 [C-2′ (H)], 122.59 [C-2′ (B)], 122.79 [C-2″/6″ (J)], 122.82–123.34 [C-5′ (B, E, H)], 123.18 [C-2′ (E)], 123.31 [C-6′ (H)], 125.46 [C-6′ (B)], 125.56 [C-5′ (E)], 127.63 [C-1″ (J)], 135.01 [C-1′ (H)], 135.19 [C-1′ (B)], 135.65 [C-1′ (E)], 138.80 [C-4″ (J)], 141.69–142.91 [C-3′/4′ (B, E, H)], 143.06 [C-3″/5″ (J)], 147.77 [C-5 or C-7 (G)], 147.81 [C-5 (A)], 148.10 [C-5 (D)], 148.79 [C-5 or C-7 (G)], 149.30 [C-7 (A)], 149.41 [C-7 (D)], 152.44/153.50 [C-8a (D, G)], 154.24 [C-8a (A)], 162.18 [Carboxyl-C (J)], 166.15–170.44 [–$\underline{C}O$–$\underline{C}H_3$].

2.3.11. *Epicatechin-(2β→7, 4β→8)-[epicatechin-(4β→6)]-epicatechin-(4β→8)-epicatechin* (**27**)

Compound **27** (parameritannin A1, 30 mg) was peracetylated for analytical investigation to *epicatechin-(2β→7,4β→8)-[epicatechin-(4β→6)]-epicatechin-(4β→8)-epicatechin-peracetate* (**27a**, 44 mg): $[\alpha]_D^{20} = +71.07°$ ($c = 0.16$); ESI-MS: $[M + Na]^+$ *m/z* 1973.2; $[\ ]_{230}$ 140162, $[\ ]_{255}$ 15355, $[\ ]_{275}$ 35441; ^{1}H NMR ($CDCl_3$, 600 MHz): δ 1.48–2.35 [3H, all *s*, aliphatic and phenolic –OAc], 2.94–3.12 [*m*, H-4a,b (I)], 4.37 [*brs*, H-4 (F)], 4.63 [*brs*, H-4 (L)], 4.73 [*d*, *J* = 4.2 Hz, H-4 (C)], 5.09 [*brs*, H-2 (I)], 5.11 [*d*, *J* = 4.2 Hz, H-3 (C)], 5.13 [*m*, H-3 (F)], 5.45 [*brs*, H-2 (F)], 5.46 [*m*, H-3 (I)], 5.54 [*m*, H-3 (L)], 5.67 [*brs*, H-2 (L)], 6.50 [*s*, H-6 (G)], 6.57 [*d*, *J* = 2.2 Hz, H-6 or H-8 (A)], 6.62 [*d*, *J* = 2.3 Hz, H-6 or H-8 (J)], 6.72 [*d*, *J* = 2.3 Hz, H-8 or H-6 (J)], 6.80 [*d*, *J* = 2.2 Hz, H-8 or H-6 (A)], 7.07 [*dd*, *J* = 2.0 and 8.4 Hz, H-6′ (H)], 7.09 [*d*, *J* = 2.0 Hz, H-2′ (H)], 7.11 [*d*, *J* = 8.4 Hz, H-5′ (H)], 7.13 [*d*, *J* = 8.4 Hz, H-5′ (K)], 7.14 [*d*, *J* = 8.4 Hz, H-5′ (B)], 7.19 [*d*, *J* = 8.4 Hz, H-5′ (E)], 7.26 [*dd*, *J* = 2.0 and 8.4 Hz, H-6′ (K)], 7.29 [*d*, *J* = 2.0 Hz, H-2′ (K)], 7.43 [2 × *d*, *J* = 2.0 Hz, H-2′ (B) and (E)], 7.50 [*dd*, *J* = 2.0 and 8.4 Hz, H-6′ (E)], δ 7.55 [*dd*, *J* = 2.0 and 8.4 Hz, H-6′ (B)]; ^{13}C NMR ($CDCl_3$, 125 MHz): δ 19–21 [–CO–$\underline{C}H_3$], 26.33 [C-4 (I)], 27.67 [C-4 (C)], 32.67 [C-4 (L)], 33.36 [C-4 (F)], 66.42 [C-3 (I)], 67.91 [C-3 (C)], 69.72 [C-3 (F)], 69.85 [C-3 (L)], 74.15 [C-2 (L)], 75.29 [C-2 (F)], 76.76 [C-2 (I)], 98.88 [C-2 (C)], 106.64 [C-6 or C-8 (A)], 107.20 [C-6 or C-8 (J)], 107.68 [C-8 or C-6 (J)], 108.79 [C-8 (D)], 109.08 [C-4a (D)], 109.23 [C-6 (D)], 110.24 [C-8 or C-6 (A)], 110.34 [C-4a (G)], 111.04 [C-6 (G)], 113.52 [C-4a (A)], 114.09 [C-4a (J)], 118.32 [C-8 (G)], 121.53 [C-2′ (H)], 122.01 [C-2′ (B) or C-2′ (E)], 122.62 [C-2′ (E) or C-2′ (B)], 122.75–123.18 [C-5′ (B, H, K)], 123.25 [C-5′ (E) and C-6′ (H)], 124.43 [C-2′ (K)], 125.27 [C-6′ (B) or C-6′ (E)], 125.35 [C-6′ (E) or C-6′ (B)], 126.02 [C-6′ (K)], 134.51–135.16 [C-1′ (B, E, H)], 136.91 [C-1′ (K)], 141.40–143.14 [C-3′/4′ (B, E, H, K)], 147.42 [C-7 (G)], 147.77 [C-7 (D)], 148.68 [C-5 (G)], 148.94 [C-5 (A)], 149.01 [C-5 (D)], 149.80 [C-7 (A)], 150.18 [C-7 or C-5 (J)], 150.24 [C-5 or C-7 (J)], 151.53 [C-8a (D)], 151.81 [C-8a (G)], 153.44 [C-8a (A)], 154.41 [C-8a (J)], 168–172 [–$\underline{C}O$–$\underline{C}H_3$].

2.3.12. *1-O-β-D-(2,4-dihydroxy-6-methoxyphenyl)-6-O-(4-hydroxy-3,5-dimethoxybenzoyl)-glucopyranoside* (**28**)

ESI-MS: $[M + Na]^+$ *m/z* 521.2; *m/z* 1018.8 $[2\ M + Na]^+$; ^{1}H NMR (MeOD, 400 MHz): δ 3.39–3.48 [*m*, H-4, H-3 and H-2], 3.65 [*m*, H-5], 3.69 [3H, *s*, $-OCH_3$], 3.88 [2×3H, *s*, $-OCH_3$], 4.38 [*dd*, *J* = 7.1 and 12.0 Hz, H-6a], 4.58 [*d*, *J* = 7.5 Hz, H-1], 4.69 [*dd*, *J* = 2.0 and 12.0 Hz, H-6b], 5.90 [*d*, *J* = 2.7 Hz, H-3′], 5.96 [*d*, *J* = 2.7 Hz, H-5′], 7.33 [2H, *s*, H-2″/6″]; ^{13}C NMR (MeOD, 100 MHz): δ 56.51 [$-OCH_3$], 56.95 [2 × $-OCH_3$], 65.27 [C-6], 71.84 [C-4], 75.25 [C-2], 76.21 [C-5], 77.56 [C-3], 93.15 [C-5′], 96.84 [C-3′], 107.61 [C-1], 108.41 [C-2″/6″], 121.29 [C-1″], 128.84 [C-1′], 142.09 [C-4″], 148.95 [C-3″/5″], 152.29 [C-2′], 154.73 [C-6′], 156.40 [C-4′], 167.98 [C-7″].

2.3.13. *Epicatechin-(2β→7, 4β→8)-epicatechin-(4β→8)-phloroglucinol* (**29**)

Degradation of 20 mg **23** with 30 mg phloroglucinol in 2 ml 1% ethanolic HCl yielded epicatechin (**2**) and **29**, which were purified using a Sephadex® LH-20 column (25 × 80 mm) with first 300 ml EtOH, then 300 ml MeOH. Compound **29** was peracetylated for analytical investigation to epicatechin-(2β→7, 4β→8)-epicatechin-(4β→8)-phloroglucinol-peracetate (**29a**): $[\alpha]_D^{20} = +106.82°$ ($c = 0.44$); ESI-MS: $[M + Na]^+$ *m/z* 1127.5.; $[\Theta]_{210}$ -34546, $[\Theta]_{230}$ 67077, $[\Theta]_{250}$ 5536, $[\Theta]_{270}$ 22775, $[\Theta]_{284}$ -3387; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.26–2.33 [*m*, aliphatic and aromatic OAc], 4.42 [*d*, *J* = 3,2 Hz, H-4 (F)], 4.60 [*d*, *J* = 4,2 Hz, H-4 (C)], 5.01 [*d*, *J* = 4,2 Hz, H-3 (C)], 5.02 [*dd*, *J* = 1,6 and 3,2 Hz, H-3 (F)], 5.52 [*d*, *J* = 1,6 Hz, H-2 (F)], 6.50 [*d*, *J* = 2,4 Hz, H-6 (A)], 6.55 [*s*, H-6 (D)], 6.84 [*d*, *J* = 2.4 Hz, H-4/6 (G)], 6.85 [*d*, *J* = 2,4 Hz, H-8 (A)], 6.94 [*d*, *J* = 2.4 Hz, H-6/4 (G)], 7.13 [*d*, *J* = 8.2 Hz, H-5′ (E)], 7.20 [*dd*, *J* = 2.0 and 8,2 Hz, H-6′ (E)], 7.26 [*d*, *J* = 2.0 Hz, H-2′ (E)], 7.27 [*d*, *J* = 8.2 Hz, H-5′ (B)], 7.48 [*d*, *J* = 2.0 Hz, H-2′ (B)], 7.58 [*dd*, *J* = 2,0 and 8,0 Hz, H-6′ (B)]; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 27.29 [C-4 (C)], 33.82 [C-4 (F)], 67.76 [C-3 (C)], 70.14 [C-3 (F)], 75.22 [C-2 (F)], 97,75 [C-2 (C)], 104.15 [C-6 (D)], 106.71 [C-4a (F)], 107.20 [C-8 (A)], 109.70 [C-6 (A)], 113.06 [C-4a (A)], 114,39 [C-4/6 (G)], 115.24 [C-6/4 (G)], 118.59 [C-8 (D)], 120.24 [C-2 (G)], 122.82 [C-5′ (E)], 123.02 [C-2′ (B and E)], 124.74 [C-5′ (B)], 125.30 [C-6′ (B)], 126.15 [C-6′ (E)], δ 134.70 [C-1′ (E)], δ 135.23 [C-1′ (B)], δ 141.64-143.02 [C-3′/4′ (B and E)], 148.69–150.41 [C-5/7 (A and D); C-1/3/5 (G)], 152.04 [C-8a (D)]; 155.57 [C-8a (A)].

2.3.14. *Epicatechin-3-O-gallate-(4β→8)-epicatechin-3-O-gallate-phloroglucinol* (**30**)

Degradation of 18 mg **22** with 30 mg phloroglucinol in 2 ml 1% ethanolic HCl yielded **30**, and 12 which were purified using a Sephadex® LH-20 column (25 × 80 mm) with first 300 ml EtOH, then 300 ml MeOH.

Compound **30** (8 mg) was peracetylated for analytical investigation to epicatechin-3-*O*-gallate-(4β→8)-epicatechin-3-*O*-gallat-phloroglucinol-peracetate (**30a**, 11 mg): the spectroscopic values (^{1}H NMR, MS, CD) were identical with published data [9].

3. Results and discussion

The total tannin content of a water extract from the dried aerial parts of *R. acetosa* L. was determined with 3.6% by the hide powder method of Pharmacopoeia Europea calculated as pyrogallol. For a detailed investigation of the tannins a crude acetone–water (7:3) extract of the aerial parts of *R. acetosa* was partitioned between ethyl acetate (EtOAc) and water to yield fractions enriched with flavan-3-ols and low molecular weight proanthocyanidins (EtOAc extract), and oligomeric proanthocyanidins of higher molecular weight (water extract), respectively. TLC studies with different staining methods of both extracts indicated the presence of proanthocyanidins and the absence of hydrolysable tannins. The EtOAc-soluble fraction was fractionated subsequently on Sephadex® LH-20 with ethanol, methanol and an acetone–water mixture. Fractions obtained were further purified using multilayer countercurrent chromatography (MLCCC), fast centrifugal partition chromatography (FCPC), low pressure chromatography on MCI® gel CHP20P, MPLC on RP-18 material or preparative TLC of the respective peracetylated derivatives to afford compounds **1** to **28** (Figs. 1–4). Structure elucidation was performed by 1D- and 2D-NMR as free phenols or peracetylated derivatives, circular dichroism (CD), optical rotation $[\alpha]_D^{20}$, ESI-MS experiments and, in part, by partial acid-catalysed degradation with phloroglucinol. Within the structural investigations of the highly complex *R. acetosa* proanthocyanidins the native, free-phenolic compounds had to be analysed, but in some cases a peracetylation of complex fractions, of isolated free-phenolic compounds and of degradation products of complex oligomeric proanthocyanidins was necessary for effective isolation and unambiguous structural elucidation.

Compounds **1**–**3** (Fig. 1) were identified by the spectroscopic data for both, the free-phenolic compounds and their peracetates **1a**–**3a** as catechin, epicatechin and epicatechin-3-*O*-gallate, respectively [10–12]. The decreased positive Cotton effect at 235 nm in the CD spectrum and the comparatively low optical rotation + 16.4° (*c* 0.14, MeOH) of compound **1a** indicated the presence of a mixture of (2R, 3 S)-catechin and (2 S, 3R)-*ent*-catechin. Quantitative CE-analysis of **1** confirmed the presence of both, catechin and *ent*-catechin in a 60:40 ratio. The presence of both enantiomers in one plant is in many cases not investigated in detail [13] especially if the biosynthetic pathway towards (+)-catechin and (−)-epicatechin is considered as the main stream.

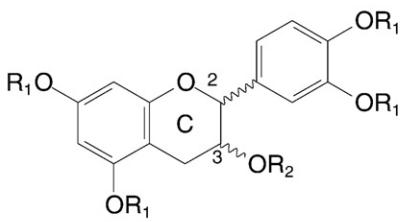
The values for the optical rotation as well as the CD spectra of compounds **2a** and **3a** were consistent with literature data [14,15] and to those of authentic reference samples. Thus, compounds **2** and **3** were confirmed as epicatechin and epicatechin-3-O-gallate.

Compounds **4**–**7** and **13**–**15** (Figs. 1 and 2) were transferred after isolation of the free penolic compounds to the respective peracetates and subsequently identified as the peracetates of the dimeric procyanidins B1 (epicatechin-(4β→8)-catechin, **4**) B2 (epicatechin-(4β→8)-epicatechin, **5**), B3 (catechin-(4α→8)-catechin, **6**), B4 (catechin-(4α→8)-epicatechin, **7**), A2 (epicatechin-(2β→7, 4β→8)-epicatechin, **15**), B5 (epicatechin-(4β→6)-epicatechin, **14**) and B7 (epicatechin-(4β→6)-catechin, **15**) by comparison of the spectroscopic data (^{1}H NMR-, ESI-MS- and CD spectra, optical rotation) of the peracetylated derivatives **4a**–**7a** and **13a**–**15a** with published data [11,16,17,33]. Extensive investigation of the proton chemical shifts in comparison with the values of peracetylated synthetic procyanidin diastereoisomers [18] showed that *ent*-catechin is not a "lower" part of these procyanidins, because such dimers will show significant shifts within the heterocyclic and A-ring protons. Further dimeric proanthocyanidins (Fig. 2) are epiafzelechin-(4β→8)-epicatechin (**8**), epiafzelechin-3-*O*-gallate-(4β→8)-epicatechin-3-*O*-gallate (**10**), epicatechin-(4β→8)-epicatechin-3-*O*-gallate (**11**), epicatechin-3-*O*-gallate-(4β→8)-epicatechin-3-*O*-gallate (**12**), epicatechin-(4β→6)-epicatechin-3-*O*-gallate (**17**) and epicatechin-3-*O*-gallate-(4β→6)-epicatechin-3-*O*-gallate (**18**). The spectroscopic data of the peracetates **11a**, **12a** and **18a** correlated well with published values [16,19–25]. Due to the lack of reference data for the peracetylated compounds **8a**, **10a** and **17a** we here report the complete NMR data set for these derivatives in detail.

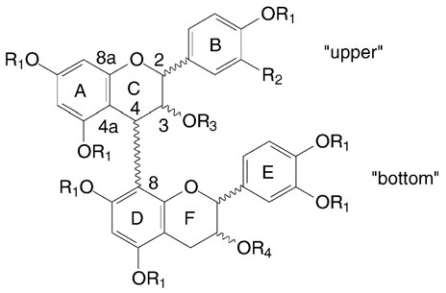
Known trimeric proanthocyanidins from *R. acetosa* were transferred after isolation to the respective peracetates and subsequently identified as the peracetates **20a** and **21a** of procyanidin C1 (epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin, **20**) and epicatechin-(4β→8)-epicatechin-(4β →8)-catechin (**21**) [17,23,26] (Fig. 2).

Cinnamtannin B1 (epicatechin-(2β→7, 4β→8)-epicatechin-(4β→8)-epicatechin, **23**) was characterized as free-phenolic compound **23** and as its peracetate **23a**. (Fig. 3). The NMR data of **23** were consistent with literature data published for cinnamtannin B1 [27–29]. However, the data for the peracetylated compound **23a** were consistent with the published values of the corresponding derivative of pavetannin B1 (epicatechin-(2β→7, 4β→8)-epicatechin-(4β→8)-*ent*-epicatechin) instead of cinnamtannin B1 [30]. In contrast, the NMR spectrum of **23a** was superimposable with those of the peracetate derivative of an authentic reference compound isolated from *Laurus nobilis* (unpublished results). In order to determine unambiguously the absolute configuration of the terminal flavan-3-ol, **23** was subjected to a controlled acid-catalysed degradation in the presence of phloroglucinol [7]. From the reaction mixture epicatechin (**2**) was identified (NMR, CD, $[\alpha]_D^{20}$) after peracetylation (**2a**) as a major degradation product and therefore **2** must be the 'bottom' flavan-3-ol unit (Fig. 1). Also epicatechin-(2β→7, 4β→8)-epicatechin-(4β→2)-phloroglucinol (**29**) (Fig. 3) was isolated from the reaction mixture and identified as its peracetate (**29a**) in comparison with the spectroscopic data of the same derivative performed as phloroglucinol cleavage product of cinnamtannin B1 from *L. nobilis* (unpublished results). Thus, compound **23** was confirmed to be cinnamtannin B1 and the published data [30] for the peracetylated derivative has to be revised.

Furthermore, the trimer epicatechin-3-*O*-gallate-(4β→8)-epicatechin-3-*O*-gallate-(4β→8)-epicatechin-3-*O*-gallate (**22**) was isolated (Fig. 2). This compound as well as the respective peracetate derivative **22a** showed very complex

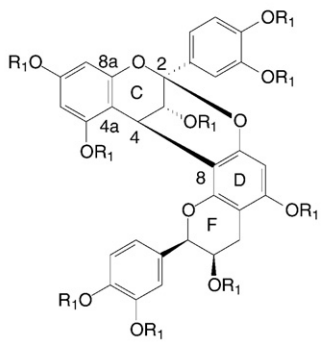


	Configuration at C-2 and C-3	R_1	R_2
1	2R, 3S	H	H
1	2S, 3R	H	H
1a	2R, 3S	Ac	Ac
1a	2S, 3R	Ac	Ac
2	2R, 3R	H	H
2a	2R, 3R	Ac	Ac
3	2R, 3R	H	G
3a	2R, 3R	H	G_{Ac}

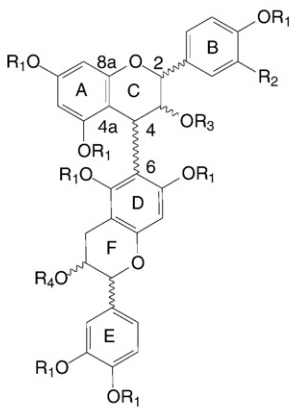


	Configuration at C-2, C-3 and C-4	Configuration at F-2 and F-3	R_1	R_2	R_3	R_4
4	2R, 3R, 4R (β)	2R, 3S	H	OH	H	H
4a	2R, 3R, 4R (β)	2R, 3S	Ac	OAc	Ac	Ac
5	2R, 3R, 4R (β)	2R, 3R	H	OH	H	H
5a	2R, 3R, 4R (β)	2R, 3R	Ac	OAc	Ac	Ac
6	2R, 3S, 4S (α)	2R, 3S	H	OH	H	H
6a	2R, 3S, 4S (α)	2R, 3S	Ac	OAc	Ac	Ac
7	2R, 3S, 4S (α)	2R, 3R	H	OH	H	H
7a	2R, 3S, 4S (α)	2R, 3R	Ac	OAc	Ac	Ac
8	2R, 3R, 4R (β)	2R, 3R	H	H	H	H
8a	2R, 3R, 4R (β)	2R, 3R	Ac	H	Ac	Ac
9	2R, 3R, 4R (β)	2R, 3R	H	H	H	G
9a	2R, 3R, 4R (β)	2R, 3R	Ac	H	Ac	G_{Ac}
10	2R, 3R, 4R (β)	2R, 3R	H	H	G	G
10a	2R, 3R, 4R (β)	2R, 3R	Ac	H	G_{Ac}	G_{Ac}
11	2R, 3R, 4R (β)	2R, 3R	H	OH	H	G
11a	2R, 3R, 4R (β)	2R, 3R	Ac	OAc	Ac	G_{Ac}
12	2R, 3R, 4R (β)	2R, 3R	H	OH	G	G
12a	2R, 3R, 4R (β)	2R, 3R	Ac	OAc	G_{Ac}	G_{Ac}

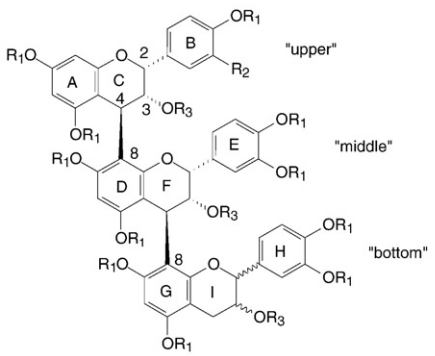
Fig. 1. Structural features of proanthocyandidins **1** to **12** isolated from *R. acetosa* and the respective peracetate derivatives produced from the free-phenolic compounds.



	R_1
13	H
13a	Ac



	Configuration at C-2, C-3 and c-4	Configuration at F-2 and F-3	R_1	R_2	R_3	R_4
14	2R, 3R, 4R (β)	2R, 3R	H	OH	H	H
14a	2R, 3R, 4R (β)	2R, 3R	Ac	OAc	Ac	Ac
15	2R, 3R, 4R (β)	2R, 3S	H	OH	H	H
15a	2R, 3R, 4R (β)	2R, 3S	Ac	OAc	Ac	Ac
16	2R, 3R, 4R (β)	2R, 3R	H	H	H	G
16a	2R, 3R, 4R (β)	2R, 3R	Ac	H	Ac	G_{Ac}
17	2R, 3R, 4R (β)	2R, 3R	H	OH	H	G
17a	2R, 3R, 4R (β)	2R, 3R	Ac	OAc	Ac	G_{Ac}
18	2R, 3R, 4R (β)	2R, 3R	H	OH	G	G
18a	2R, 3R, 4R (β)	2R, 3R	Ac	OAc	G_{Ac}	G_{Ac}



	Configuration at I-2 and I-3	R_1	R_2	R_3
19	2R, 3R	H	H	H
19a	2R, 3R	Ac	H	Ac
20	2R, 3R	H	OH	H
20a	2R, 3R	Ac	OAc	Ac
21	2R, 3S	H	OH	H
21a	2R, 3S	Ac	OAc	Ac
22	2R, 3R	H	OH	G
22a	2R, 3R	Ac	OAc	G_{Ac}

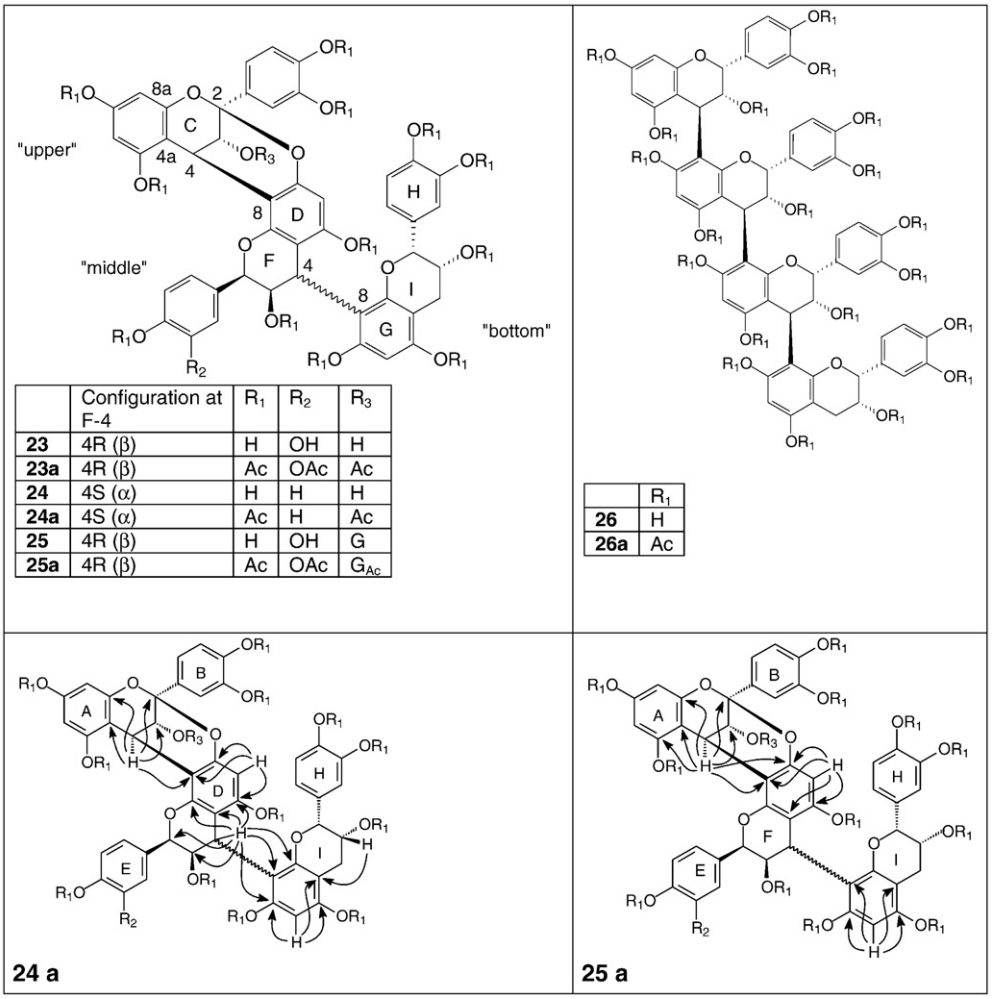
Fig. 2. Structural features of compounds **13** to **22** isolated from *R. acetosa* and the respective peracetate derivatives produced from the free-phenolic compounds.

spectra in ambient and low temperature (243 K) NMR experiments [44] so that a complete assignment of signals failed. Therefore **22** was hydrolyzed by acid cleavage in the present of phloroglucinol in analogy to the experiments performed with compound **23**. The resulting cleavage products were identified (NMR, MS, CD, $[\alpha]_D^{20}$) as the corresponding peracetate derivatives **12a** and **30a** of epicatechin-3-*O*-gallate-(4β→8)-epicatechin-3-*O*-gallate (**12**) and epicatechin-3-*O*-gallate-(4β→8)-epicatechin-3-*O*-gallate-(4β→2)-phloroglucinol (**30**). Thus, the structure of **22** was deduced from these cleavage products.

Tetrameric proanthocyanidins were identified (Fig. 3) as their peracetates **26a** and **27a** with the structural features of procyanidin D1 (epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin, 26) and parameritannin A1 (epicatechin-(2β→7,4β→8)-(epicatechin-(4β→6))-epicatechin-(4β→8)-epicatechin, **27**) in comparison with literature data [27,31]. Structure elucidation of the later peracetate (**27a**) was also confirmed by the comparison of its spectroscopic data with a reference sample isolated from *L. nobilis* (unpublished results).

A more detailed description is made on the structural features of compounds **9**, **16**, **19**, **24**, **25** and **28** which were found to the best of our knowledge to be new natural products.

Compound **9** was characterized after acetylation as its peracetate **9a**. The ESI-MS pseudomolecular ion ($[M+Na]^+$ *m/z* 1199.5) of 9a indicated the presence of a monogalloylated dimeric proanthocyanidin with an (epi)catechin and an (epi)afzelechin unit. ^1H NMR in $CHCl_3$ revealed its close structural resemblance to that of the corresponding derivative of epiafzelechin-(4β→8)-epicatechin (**8a**). To prove the 4→8 interflavan linkage in **9a** indirect shift parameters were used. The chemical shift of the A-ring protons (δ 6.12 and 6.50 ppm; [32]) and the strong dominance of one rotamer (*ca* 3–5:1; [7]) correlated well with published data for 4→8 linked proanthocyanidins. In contrast to compound **8a**, signals in **9a** for H-2 (F) and H-3 (F) were shifted downfield (*ca* Δδ 0.2 ppm), probably due to the substitution of the hydroxyl group at C-3 (F) with gallic acid. Unfortunately, no direct proof for the point of attachment was visible in the HMBC spectrum due to the lack of a correlation between the carboxyl carbon and the respective H-3 proton of the "upper"



	Configuration at F-4	R_1	R_2	R_3
23	4R (β)	H	OH	H
23a	4R (β)	Ac	OAc	Ac
24	4S (α)	H	H	H
24a	4S (α)	Ac	H	Ac
25	4R (β)	H	OH	G
25a	4R (β)	Ac	OAc	G_{Ac}

	R_1
26	H
26a	Ac

Fig. 3. Structural features of compounds **23** to **27**, **29** and **30** isolated from *R. acetosa* and the respective peracetate derivatives produced from the free-phenolic compounds; key correlations in the 2D-NMR (HMBC) are marked with arrows for **24a** and **25a**.

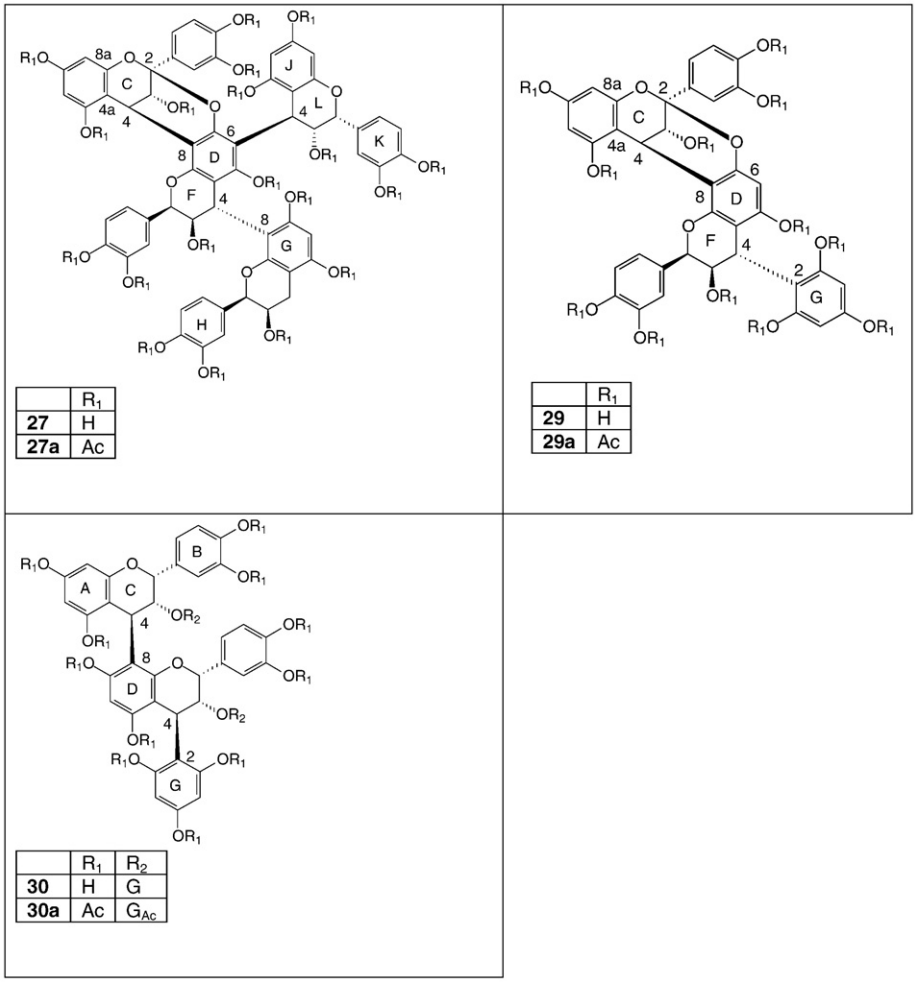


Fig. 3 (*continued*).

or "bottom" flavan-3-ol unit. However, further evidence for the point of acylation was deduced by the ^{1}H NMR spectra of peracetylated dimeric proanthocyanidins from the observation that the chemical shift of the two-proton singlet of the gallic acid moiety obviously depends on where the esterification has taken place: protons of a galloyl moiety located at the C-3 hydroxyl of the "bottom"-units are found to have resonances at δ7.64–7.72 ppm, while substitution at C-3 of the "upper"-units are monitored at δ7.50–7.60 ppm [43], see also compounds **10**–**12**, **17**, **18**). Therefore, the two-proton

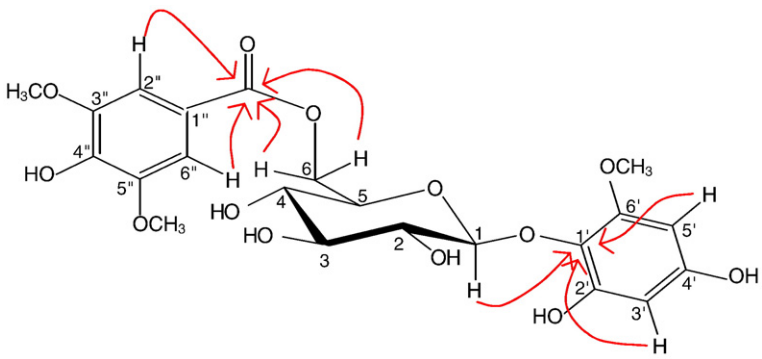


Fig. 4. Structure of 1-*O*-β-D-(2,4-dihydroxy-6-methoxyphenyl)-6-*O*-(4-hydroxy-3,5-dimethoxybenzoyl)-glucopyranoside **28**; key correlations in the 2D-NMR (HMBC) are marked with arrows.

resonance at δ 7.64 ppm in **9a** indicated "bottom" acyl substitution of 9. Within HMBC experiments the correlation of H-2 (C) to the C-2′/6′ signals of the monohydroxylated B ring provide the "upper"-unit as epiafzelechin. HMBC correlations of protons H-6 (D) with C-7 (D), C-5 (D), C-8 (D) and C-4a (D) indicated again the existence of a 4→8 linkage. The relative 2,3-*cis* configuration of the flavan-3-ol units was obvious from the small coupling constants of all heterocyclic protons ($J \leq 2$ Hz). The 4*R* configuration was deduced from the strong positive Cotton effect within 200–240 nm in the CD spectrum of **9a** [11]. Thus, **9** was deduced to be epiafzelechin-(4β→8)-epicatechin-3-*O*-gallate, a new natural product.

Compound **19**, after peracetylation (**19a**) showed a pseudomolecular ion $[M+Na]^+$ at *m/z* 1461.5 indicating the presence of two (epi)catechin and one (epi)afzelechin flavan-3-ol unit. The small coupling constants of the heterocyclic protons ($J \leq 2$ Hz) in the ^{1}H NMR spectrum of **19a** indicates the relative 2,3-*cis* configuration of all the flavan-3-ol units. Within HMBC experiments the signals of H-2 (C), H-2 (F) and H-2 (I) coupled to the respective C-1′ signals of the aromatic rings B, E and H. Long-range connectivities (HMBC) between the H-3′/5′ (B) protons and the carbon C-1′ (B) determined the "upper"-unit to be epiafzelechin. The comparison of the ^{1}H NMR spectrum of **19a** with that of procyanidin C1 (20a) proves the strong resemblance except for an AA′BB′ spin-system in 19a instead of an AMX-spin-system in 20a. The high amplitude positive Cotton effect in the 200–240 nm region of the CD spectrum indicated the configuration at both interflavan linkages to be 4*R*. Consequently, the structure of **19** was deduced to be epiafzelechin-(4β→8)-epicatechin-(4β→8)-epicatechin, a new natural product.

Compound **16** was characterized after acetylation as its peracetate **16a**. The ESI-MS pseudomolecular peak *m/z* 1199.2 ($[M+Na]^+$) for **16a** indicated the presence of a monogalloylated dimeric proanthocyanidin with an (epi) catechin and an (epi)afzelechin unit.

Due to broadening signals caused by of rotational isomerism the ^{1}H NMR spectrum of **16a** at ambient temperature and the similarity to the spectrum of **17a** indicated the presence of a 4→6 linkage [7]. The downfield shift of both the A-ring proton resonance (δ 6.74 ppm, H-8) and the H-2 (F) (δ 5.26 ppm) confirmed the 4→6 interflavanoid linkage [32,33]. The substitution of gallic acid to the "bottom" unit was shown again by the typical resonance for the two-proton singlet at δ 7.64 ppm as argued above (see compound **9**). Unfortunately the yield of **16a** was to low to perform ^{13}C NMR and 2D NMR experiments. To clarify the sequence of the two flavan-3-ol units, oxidative cleavage of the C–C interflavan linkage under acidic conditions was performed. Under the conditions of this anthocyanidin reaction the C–C interflavan linkages will be cleaved and coloured anthocyanidium cations will be released, which can be identified easily after TLC separation against the respective reference compounds. During this experiment the resulting cleavage product from the free-phenolic dimer **16** was identified by TLC and respective reference compounds to be pelargonidin, originating from the former "upper" flavan-3-ol unit. The high amplitude positive Cotton effects at low wavelength in the CD spectrum of **16a** confirmed the absolute configuration as 4*R*. Thus, the structure of **16** was deduced to be epiafzelechin-(4β→6)-epicatechin-3-*O*-gallate, also a new natural product.

The ESI-MS of compound **24** after acetylation in form of its peracetate **24a** gave a pseudomolecular ion $[M+Na]^+$ at *m/z* 1417.6 indicating the presence of a trimeric A-type procyanidin composed of two (epi)catechin units and one (epi) afzelechin unit. 1D- and 2D-NMR spectra of **24a** were similar to those obtained from cinnamtannin B1 (**23a**). The HMBC spectrum of **24a** is given in Fig. 3 and displayed for the proton H-4 (C), which could be assigned to the A-type-linked flavan-3-ol, and H-8 (A) a coupling to the carbon C-8a (A) of the "upper" unit. This shows that the additional A-type linkage is located between the "upper" and the "middle" flavan-3-ol. The presence of all 4→8 linkages was proven by the coupling of H-6 (D/G) to the respective signals of C-5 (D/G) and C-7 (D/G). H-2 (F) shows a ^3J-coupling to the C-2′/6′ of the epiafzelechin unit which means that the 1,4-disubstituted aromatic ring is connected to the "middle" flavan-3-ol unit. The coupling constants of the proton signals of the "middle" unit ($J_{3,4} = 4{,}8$ Hz; $J_{2,3} = 2{,}2$ Hz) are to high for a typical 2,3-*cis*-3,4-*trans*-configuration, but to low for a 2,3-*trans*-3,4-*trans*-configuration. According to Schlepp et al. [35] such coupling constants are typical for 4→8 α-linked 2,3-*cis*-flavan-3-ols. To investigate the exact orientation of the interflavan linkage 1D ROESY-NMR experiments were performed and compared with data obtained from at position C-4 (F) β-configurated cinnamtannin B1 (**23a**). Irradiation of H-2 (I) from the cinnamtannin B1 resulted in signals from H-6 (D), H-3 (C) and H-2 (F). In contrast to that no signals of these protons were observed in case of irradiation of H-2 (I) from **24a**. This indicates α-orientation of the interflavan linkage. The CD spectrum of compound **24a** showed a decreased positive Cotton effect between 200 and 240 nm compared to the CD spectrum of compound **23a**. This is another hint for the *alpha*-orientation of the interflavan linkage. However, an *ent*-configuration of the epiafzelechin unit cannot be excluded. Thus, the structure of **24** can tentatively be described as epicatechin-(2β→7, 4β→8)-epiafzelechin-(4α→8)-epicatechin, a new natural product.

Compound **25** was characterized after acetylation as its peracetate **25a**. The ESI-MS pseudomolecular ion *m/z* 1711.3 $[M+Na]^+$ agreed with a monogalloylated trimeric A-type procyanidin. Within HMBC experiments (Fig. 3) the signal of H-4 (C) (δ 4.21 ppm), which correlates to a flavan-3-ol unit with A-type linkage, coupled to the C-4a of the "upper" unit. The same carbon connectivity was due for H-8 (A). This means that the ether bridging system must be located between the "upper" and "middle" flavan-3-ol units.

The 4→8 linkage of all three flavan-3-ols was deduced by the coupling of signals from H-6 (D/G) to C-5 and C-7 (D/G). The galloylation of the "upper" flavan-3-ol unit was evident from the typical signal at δ 7.21 ppm and by the ^3J-coupling of H-2″/6″ (*J*) and H-3 (C) to the carboxyl group of the gallic acid moiety. The course of the CD spectrum and the optical rotation values were comparable to those measured for **23a**. For that identical stereochemical properties of **25** and cinnamtannin B1 (**23**) can be deduced, leading to the structural features for **25** to be epicatechin-3-*O*-gallate-(2β→7, 4β→8)-epicatechin-(4β→8)-epicatechin. So far, galloylated A-type proanthocyanidins have not been described before.

A polymeric procyanidin fraction was obtained from the aqueous phase of the water–EtOAc partition after elution from Sephadex® LH20 according [34] an average degree of

polymerisation with 7 to 8 flavan-3-ol units [19,26,38] by using the ratio of the signals of C-3 of the terminal unit at δ 67 ppm and those of the C-3 carbons of the extender flavan-3-ol units at δ 73 ppm. The dominance (*ca* 5:1) of procyanidin residues over the propelargonidin units was deduced by the intensity of the respective signals of C-3′ and C-4′ resonances of the 1,3,4-trisubstituted B rings (δ 145 ppm) and the C-4′ of the 1,4-substituted analogues at δ 157 ppm. Flavan-3-ol residues within the polymer chain were mainly 2,3-*cis* configurated (typical signal of C-2 at δ 76 ppm) [36,39]. A signal indicating the presence of 2,3-*trans* units at δ 79 ppm was below the limit of quantitation. 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 [37].

Compound **28** was initially regarded to be a proanthocyanidin or flavan-3-ol-glycoside due to its red coloured spot on TLC after vanillin/HCl spray detection and its typical UV_{max} around 274 nm. However, the elution before monomeric flavan-3-ols from the Sephadex® LH-20 column and identical chromatographic behaviour were observed for phloroglucinolglycosides [40]. The ^1H NMR in MeOD indicated signals typical for carbohydrate residues (δ 3.39–4.69 ppm), also typical for an aromatic two-proton singlet (δ 7.33 ppm) and a methoxy substitution pattern (δ 3.69 and δ 3.88 ppm). Within the HMBC spectrum long-range correlations (3 J) between the H-6 protons and a carboxylic carbon (C-7″) and the anomeric proton H-1 to the C-1′ of a further aromatic ring system showed that the carbohydrate part is located between two aromatic systems (Fig. 2). Complete HMBC assignments and NOE experiments indicated one aromatic system to be 2,4-dihydroxy-6-methoxyphenol and the other one to be 4-hydroxy-3,5-dimethoxy-carboxylic acid (syringic acid). Identity of the carbohydrate part of the molecule as D-glucose was determined by capillary zone electrophoresis after hydrolysis [8] against the respective reference compounds. β-configuration of the glycosidic linkage was verified by the large coupling constant $^3J_{H\text{-}1/H\text{-}2} = 7.5$ Hz.

From these data the structure of **28** was deduced to be 1-*O*-β-D-(2,4-dihydroxy-6-methoxyphenyl)-6-*O*-(4-hydroxy-3,5-dimethoxybenzoyl)-glucopyranoside (Fig. 4), an until now unknown natural product. Similar, but not methoxylated compounds have been isolated from the proanthocyanidin rich sources *Sedum sediforme*, *Cistus* and *Rheum* species [12,40,41].

4. Conclusions

The tannin content of the aerial parts of *R. acetosa* L. was shown to be composed of a complex mixture of mono-, oligo- and polymeric proanthocyanidins consistent of procyanidins and propelargonidins. The accumulation of both, A- and B-types in this plant is obvious, also the high degree of galloylation. Glycosylated proanthocyanidins or flavan-3-ol precursors were not detected. As previous studies have shown (authors own work, unpublished results), galloylation of oligomers dramatically increases cell toxicity of the proanthocyanidins against pro- and eucariotic cells. This biosynthetic strategy of *R. acetosa* and many other Polygonaceae may be seen as an effective defense strategy. Concerning the structural features of the oligomeric proanthocyanidins it is interesting that in the B-ring monohydroxylated flavan-3-ol unit (epiafzelechin) are, except for compound **24**, always found as the "upper" flavan-3-ol unit. On the other side epiazelechin was not found as flavan-3-ol. These findings have to be discussed that in the B-ring monohydroxylated precursors are more effectively converted into the biosynthesis of oligomeric proanthocyanidins.

Spencer et al. [42] recently investigated the proanthocyanidin pattern of *Rumex obtusifolius*. The proanthocyanidin pattern of this species and *R. acetosa* seem to be similar concerning A- and B-type linked procyanidins. Even no compounds containing epiafzelechin subunits were found, the published ^{13}C-spectrum of the polymeric fraction of *R. obtusifolius* seems to be almost identical to the one recorded for *R. acetosa*. In conclusion, the proanthocyanidin pattern of the two *Rumex* species are closely related and the existence of propelargonidins also in *R. obtusifolius* may be supposed.

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