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FLAVAN-3-OLS AND PRODELPHINIDINS FROM *STRYPHNODENDRON ADSTRINGENS*

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Key Word Index—*Stryphnodendron adstringens*; Mimosaceae; stem bark; tannins; proanthocyanidins; flavan-3-ols; prodelphinidins.

Abstract—Six flavan-3-ols and eight proanthocyanidins have been isolated from the stem bark of *Stryphnodendron adstringens*, which is traditionally used in Brazil against various diseases. Two new flavan-3-ols were isolated as epigallocatechin 3-*O*-(3,5-dimethyl)-gallate and epigallocatechin 3-*O*-(3-methoxy-4-hydroxybenzoate). Isolation and elucidation of four new proanthocyanidins gave epigallocatechin-(4β → 8)-epigallocatechin 3-*O*-(4-hydroxy)benzoate epigallocatechin-(4β → 6)-epigallocatechin, gallocatechin-(4α → 8)-epigallocatechin 3-*O*-gallate and gallocatechin-(4α → 8)-epigallocatechin 3-*O*-(4-hydroxy)benzoate. So the bark of *S. adstringens* is a rich source of proanthocyanidins with biologically active pyrogallol units.

INTRODUCTION

Stryphnodendron adstringens (Martius) Coville [1] is a medicinal plant which grows in the 'Cerrados' of Brazil and is commonly known as barbatimão. The stem bark of *S. adstringens* is mentioned in the Brazilian Pharmacopoeia, with a content of at least 20% tannins [2]. Decoctions or infusions of the crude drug are traditionally used by the native population of Brazil against leukorrhoea, diarrhoea, and as an anti-inflammatory agent [3,4]. As the composition of flavan-3-ols and proanthocyanidins as constituents of the tannins has not yet been investigated, we carried out a study in order to obtain information about the composition of the tannin fraction.

RESULTS AND DISCUSSION

Multiple chromatography on Sephadex® LH-20 and multilayer coil countercurrent chromatography (MLCCC) of the ethyl acetate fraction originating from an acetone–H_2O extract of the air-dried stem bark of *S. adstringens* yielded compounds **1**–**14**. Compounds **1**, **2** and **3** were identified as gallocatechin, epigallocatechin and epigallocatechin 3-*O*-gallate by comparison of spectroscopic data of the peracetates (^{1}H NMR, $[\alpha]_D^{20^\circ}$) with authentic material.

The ^{1}H NMR spectrum ($CDCl_3$) of acetate **4a** showed similarities to that of the corresponding derivative of gallocatechin, except for a three-proton singlet at δ3.80 indicating a methoxyl group. The heterocyclic protons displayed an ABMX-system characteristic for the spin pattern of 2,3-*trans* flavan-3-ols ($J_{2,3}$ = 6.3 Hz) [5, 6]. An optical rotation of $[\alpha]_D^{20^\circ} + 28^\circ$ (acetone–H_2O 1:1; *c* 0.2) verified the 2*R*,3*S* absolute configuration of **4a**. The DCI mass spectrum showed a prominent peak $[M + 18]^+$ at *m*/*z* 548. The methoxyl group was set at the C-4′ position on the basis of the results of a COLOC experiment, which showed correlations between C-4′ (δ144.3) and both H-2′/6′ (δ6.97) and the methoxyl protons at δ3.80. This arrangement was confirmed by negative NOE interaction, when the methoxyl protons were irradiated [7]. Thus, **4** was identified as 4′-*O*-methylgallocatechin, recently described for *Panda oleosa* (Pandaceae) [8].

Separation of **5** and **6** could not be achieved with column chromatography on Sephadex® LH-20 or MLCCC (see Experimental). Normal-phase HPLC with a cyano-silica packing offered a good separation of the peracetylates under conditions described in [9]. Structures of **5** and **6** were established on the basis of the spectral data (^{1}H NMR, ^{1}H–H COSY, NOE difference spectrum) of their peracetates. The ^{1}H NMR data of **5a** and **6a** in $CDCl_3$, along with considerations on the structures of the closely related acetates of **2** and **3**, suggested **5** and **6** with an epigallocatechin basic skeleton as well as an acyl unit located at 3-*O*-position to which one (**6**) or two (**5**) methoxyl groups are introduced. This was confirmed by the signals of two overlapping methoxyl singlets at δ3.79 and an additional two-proton singlet at δ7.09 in **5a**. The latter can be assigned to the chemical equivalent

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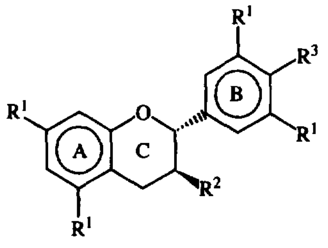
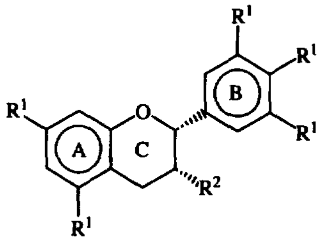
protons H-2″ and H-6″ of the acyl moiety. Compound **6a** displayed one methoxyl group at δ3.82 and an additional aromatic AMX-spin system (δ7.02–7.50) corresponding to the acyl moiety. Location of the methoxyl groups in **5a** and **6a** was confirmed by NOE difference experiments run in acetone-d_6. Irradiation of the overlapping methoxyl signals (δ3.79) in **5a** induced a NOE at the two-proton singlet H-2″/6″ (δ7.09), whereas a strong NOE was observed for the protons H-2′/6′ when a resonance at δ5.59 (H-2) was irradiated. This indicates the methoxyl groups at C-3″ and C-5″ position of the acyl moiety. Irradiation at the frequency of the methoxyl group at δ3.82 for **6a** induced a strong NOE of the H-2″ signal (δ7.44) indicating the methoxyl group at C-3″ position. This is supported by the significant NOE association between H-2(C) and the two-proton singlet H-2′/6′. The downfield shift (*ca* Δ 0.27 ppm) of the H-3(C) protons in $CDCl_3$ (δ5.65 and 5.64 in **5a** and **6a**, respectively) in comparison with that of epigallocatechin-peracetate (δ5.38) proved the attachment of the acyl moieties at the C-3 hydroxyl. The heterocyclic coupling constant ($J_{2,3}$ < 2 Hz) confirmed a relative 2,3-*cis* configuration in each case. The 2*R*,3*R* configuration was assigned to **5a** and **6a** based on the negative $[\alpha]_D^{20^\circ}$ values (−24.7°, acetone, *c* 0.15; and −27°, acetone, *c* 0.1). The proposed structures are supported by DCI mass spectral data, which exhibit a prominent $[M + 18]^+$ peak at m/z 756 (**5a**) and 726 (**6a**). Acid hydrolysis gave syringic acid (**5a**) and vanillic acid (**6a**), identified by co-chromatography (TLC) with authentic compounds. Accordingly, **5** and **6** are the new epigallocatechin 3-*O*-(3,5-dimethyl)gallate and epigallocatechin 3-*O*-(3-methoxy-4-hydroxy)benzoate.

The prodelphinidins **7**, **8** and **9** were identified as epigallocatechin-(4β → 8)-gallocatechin, epigallocatechin-(4β → 8)-epigallocatechin and epigallocatechin-(4β → 8)-epigallocatechin 3-*O*-gallate by comparison of the physical data (^{1}H NMR, DCI-MS, CD) of their peracetates **7a**, **8a** and **9a** with recently published data [10, 11].

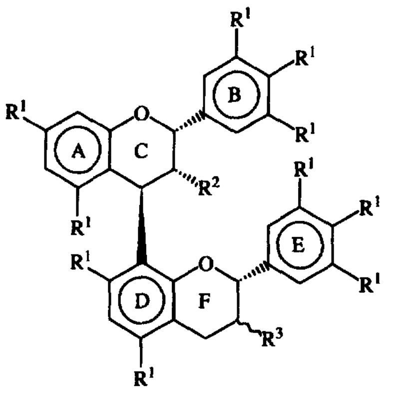
Structural assignment of the new natural prodelphinidin **10** was achieved by comparison with the closely related dimer **9**. Instead of the two-proton galloyl singlet at δ7.71 in the ^{1}H NMR spectrum of **9a**, A_2B_2-type aromatic signals were found at δ7.90 and 7.06 in **10a**, indicating a *p*-hydroxybenzoyl group. The H-3(F) proton (δ5.28) of **10a** showed a significant downfield shift (Δ 0.17 ppm) in comparison with the equivalent proton of the acetate **8a** (δ5.11); therefore, the *p*-hydroxybenzoyl group was considered to be located at the C-3(F) hydroxyl. Supporting evidence for the presence of the acyl moiety and the pyrogallol type B- and E-ring oxygenation pattern was obtained from acid hydrolysis and



	R¹	R²	R³
4	OH	OH	OMe
4a	OAc	OAc	OMe



	R¹	R²
5	OH	O-(3,5-dimethylgalloyl)
5a	OAc	O-(3,5-dimethyl-4-acetoxygalloyl)
6	OH	O-(3-methoxy-4-hydroxybenzoyl)
6a	OAc	O-(3-methoxy-4-acetoxybenzoyl)



	R¹	R²	R³
7	OH	OH	◀ OH
7a	OAc	OAc	◀ OAc
8	OH	OH	····· OH
8a	OAc	OAc	····· OAc
9	OH	OH	····· Ogalloyl
9a	OAc	OAc	····· Ogalloylperacetate
10	OH	OH	····· O(4-OHbenzoyl)
10a	OAc	OAc	····· O(4-OAcbenzoyl)
11	OH	Ogalloyl	····· Ogalloyl
11a	OAc	Ogalloyl-peracetate	····· Ogalloylperacetate

DCI-mass spectrometry ($[M + 18]^+$ m/z 1252) of the acetate. The heterocyclic coupling constant ($J_{2,3} < 2$ Hz) confirmed the relative 2,3-*cis* configuration of the 'upper' and 'lower' constituent units. Chemical shifts for H-2(F) (δ4.66) [12], H-6(A) (δ6.17) and H-8(A) (δ6.27) [13], in conjunction with the dominance of one conformer [14], indicated a (4 → 8) interflavanoid linkage. In contrast to the expected positive Cotton effect in the CD spectrum for 4β-linked dimers [15, 16], the negative Cotton effect in the 220–240 nm wavelength region of **10a** was in agreement with those observed for the acetates of 3′-*O*-acylated 4 → 8-linked proanthocyanidin dimers with a relative 2,3-*cis*-3,4-*trans* configuration [11]. The same reversal of the Cotton effect for the peracetates of the monomeric epigallocatechin 3-*O*-gallate and of epigallocatechin is already known [17]. Thus, **10** was identified as epigallocatechin-(4β → 8)-epigallocatechin 3-*O*-*p*-hydroxybenzoate.

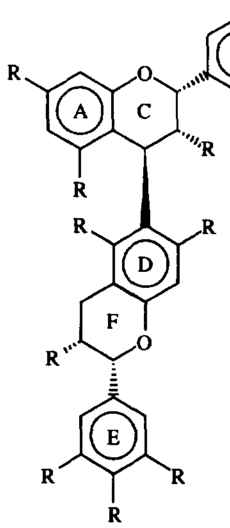
Identification of epigallocatechin 3-*O*-gallate-(4β → 8)-epigallocatechin 3-*O*-gallate (**11**), characterized several times in the literature in underivatized phenolic form [18–21] was effected by comparison of the ^{1}H NMR spectral data of its peracetate **11a** with those of the analogous derivatives **8a** and **9a**. Four sharp low-field two-proton singlets at δ7.08, 7.22, 7.56, and 7.75 indicated the chemical equivalent protons of the E- and B-ring and the presence of two galloyl groupings. Supporting evidence for the presence of galloyl moities and the pyrogallol type B- and E-ring oxygenation pattern obtained from acid hydrolysis and DCI-mass spectrometry $[M + 18]^+$ m/z 1605 of the acetate were applied. The downfield shift (Δ 0.53 ppm) of the H-3(C) (δ5.64) and (Δ 0.16 ppm) of H-3(F) (δ5.27) in the ^{1}H NMR spectrum of **11a** in comparison with the equivalent protons in **8a** suggested that the galloyl moities were located at the C-3(C) and C-3(F) position. The chemical shift of the B-ring two-proton singlet at δ7.22 indicated a 4 → 8 linked dimer with a 2,3-*cis* configurated 'upper' flavanyl unit [10], while small coupling constants in the heterocyclic region ($J_{2,3}$ and $J_{3,4} < 2$ Hz) confirmed the relative 2,3-*cis*-3,4-*trans* (C):2,3-*cis* (F) stereochemistry. The 4 → 8 interflavanyl linkage was confirmed by the chemical shift criteria of the overlapping signals H-6(A) and H-8(A) at δ6.31 [13]. The 4β linkage of the flavanyl substituent and the resulting 4*R* absolute configuration in **11a** were deduced from the negative Cotton effect in the CD spectrum at 210–240 nm.

Structural similarity of epigallocatechin-(4β → 6)-epigallocatechin (**12**) and procyanidin B_5 [12] became evident from ^{1}H spectral comparison of their peracetates. The ^{1}H NMR spectra of these compounds were virtually superimposable, except for the B-ring region. Replacement of the AMX-spin systems in the procyanidin B_5-peracetate by two two-proton singlets at δ7.23 and δ7.18 clearly supported the B-ring pyrogallol hydroxylation pattern in **12a**. While the downfield position of the aromatic two-proton singlets signified a 4 → 6 interflavanyl linkage [11], the intense positive Cotton effect between 210–240 nm indicated a 4β-linked dimer with a 4*R* absolute configuration.

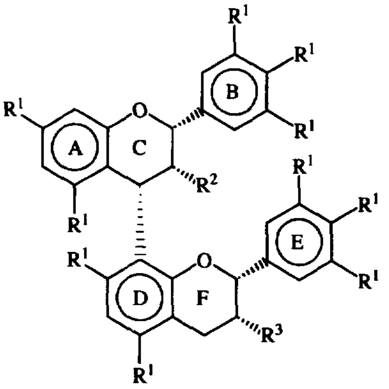
Compound **13** showed a parent ion peak at m/z 1368 $[M + 18]^+$ in the DCI-MS of the peracetate (**13a**), suggesting a monogalloylated dimeric prodelphinidin. ^{1}H NMR of **13a** in $CDCl_3$ gave three sharp two-proton singlets at δ6.76, 7.03 and 7.64, corresponding each to the equivalent protons of the E- and B-ring and a galloyl moiety. The relative all-*trans* configuration of the heterocycle C was evident from the large coupling constants ($J_{2,3} = 9.8$ Hz and $J_{3,4} = 10.0$ Hz), whereas the 2,3-*cis* stereochemistry of the 'lower' flavan unit was indicated by the small coupling constant ($J_{2,3} < 2$ Hz) of the corresponding resonances. Comparison of the ^{1}H NMR data of **13a** with those of the acetate of the parent compound gallocatechin-(4α → 8)-epigallocatechin [22–25] revealed their close structural resemblance. Because **13a** has shown a significant downfield shift (Δ 0.47 ppm) of H-3(F) (δ5.75) in comparison with the corresponding proton of the unsubstituted compound (δ5.28 [24]), the galloyl group was considered to be located at the C-3(F) hydroxyl. In combination with the negative Cotton effect in the 210–240 nm region of the CD spectrum of **13a**, the identity of **13** was established as gallocatechin-(4α → 8)-epigallocatechin 3-*O*-gallate.

The structure of the remaining prodelphinidin **14** was deduced by the comparison of the ^{1}H NMR data ($CDCl_3$) of its acetate **14a** with those of the acetate **13a**. Except for replacement of the two-proton singlet of the galloyl unit by the respective A_2B_2 aromatic spin system in **14a** the remaining parts of the spectra were remarkably similar. The point of attachment of the *p*-hydroxybenzoyl group was determined by the downfield position of H-3(F) (δ5.62) compared with the unsubstituted compound. The negative Cotton effect in the 210–240 nm region of the CD spectrum indicated a 4α-flavanyl substituent and resulted a 4*S* absolute configuration. The proposed structure was confirmed by DCI-mass spectrometry of its peracetate, which yielded a prominent ion peak at m/z 1252 $[M + 18]^+$. Acid hydrolysis of **14** under the conditions employed in [26] gave epigallocatechin 3-*O*-*p*-hydroxybenzoate (the 3-*O*-acyl ester is stable under the conditions used; see Experimental), which was identified by co-chromatography (TLC) with an authentic sample recently isolated from *Cistus salvifolius* [11]. Thus, **14** was identified as gallocatechin-(4α → 8)-epigallocatechin 3-*O*-*p*-hydroxybenzoate.

In conclusion, our research on polyflavanoids and related compounds from the stem bark of *S. adstringens* revealed the presence of new 3-*O*-esters of epigallocatechin (**5**, **6**) and new dimeric prodelphinidins (**10**, **12–14**). Compounds **10** and **14** are remarkable in that they possess epigallocatechin acylated with *p*-hydroxybenzoic acid as the terminating 'lower' unit. The crude drug is a rich source of prodelphinidins and polyphenols with a pyrogallol moiety. Proanthocyanidins exhibit antidiarrhoeic [27, 28] and anti-inflammatory [29] activity; they also show antimicrobial properties [30, 31]. Proanthocyanidins with pyrogallol units (prodelphinidins and their galloyl esters) appear to have pronounced antiviral activities [32]. The traditional use



	R
12	OH
12a	OAc



	R^1	R^1	R^3
13	OH	OH	Ogalloyl
13a	OAc	OAc	Ogalloylperacetate
14	OH	OH	O-(4-OHbenzoyl)
14a	OAc	OAc	O-(4-OAcbenzoyl)

of preparations of *S. adstringens* may be partly related to the occurrence of these compounds. Investigations on the tannins point to the presence of prorobinetinidins.

EXPERIMENTAL

General. Spray reagents, acetylation procedures, and instruments used to obtain physical data were the same as described previously [10]. DCI spectra were obtained with NH_3 as reactant gas in positive-ion mode. Analytical TLC was carried out on aluminium sheets (Kieselgel 60 F_{254}, 0.2 mm, Merck) using EtOAc–HCO_2H–H_2O (18:1:1; system S1). Peracetate spectra were recorded in $CDCl_3$, δ are given relative to TMS, unless stated otherwise. Prep. TLC was performed on silica gel plates (Kieselgel 60 F_{254}, 0.5 mm, Merck) using toluene–Me_2CO (7:3; system S2). Authentic compounds were from the collection of this Institute.

Conversion of proanthocyanidins to anthocyanidins. The proanthocyanidin (*ca* 1 mg) was refluxed with 5% HCl in EtOH for 1 hr. The reaction mixt. was subsequently chromatographed on cellulose (Cellulose F, 0.2 mm, Merck Darmstadt) using HCO_2H–HCl–H_2O (10:1:3) with delphinidin as ref. substance.

Identification of lower terminal flavan-3-ol unit. Treatment of each free phenolic proanthocyanidin (*ca* 1 mg) in 0.1 M ethanolic HCl (2 ml) at 60° for 15 min [26] liberated the respective flavan-3-ol unit which was detected by TLC on cellulose in H_2O–dioxan (10:1) or on silica gel in system 1 using ref. substances of this Institute. Hydrolysis of epigallocatechin 3-*O*-gallate and epigallocatechin 3-*O*-*p*-hydroxybenzoate did not occur under these conditions up to 6 hr.

Plant material. The stem bark of *S. adstringens* was collected in Reserva de Cerrado-FAPESP (State of São Paulo, Brazil; 22°15′S, 47°W) in March 1991 and identified with the help of Dr Sandra Maria Gomes da Costa (State of Maringá University). A voucher specimen (PBMS 73) is deposited at the Herbarium of the Institut für Pharmazeutische Biologie, Münster (Germany).

Extraction, isolation and identification of compounds. Air-dried stem bark (480 g) was extracted with Me_2CO–H_2O (7:3, 4.8 l). The combined extracts were filtered and evapd under reduced pressure to 0.5 l and lyophilized (183 g). This fr. was redissolved in 5 l H_2O

and extracted with EtOAc (27 l). After evapn of solvents, the EtOAc extract and the remaining H_2O phase gave dark brown solids of 31 g and 152 g, respectively. A portion (15 g) of the EtOAc extract was subjected to CC on Sephadex LH-20 (710 × 50 mm; eluents: 50% EtOH (5 l), EtOH (5 l), 50% MeOH (3.5 l), MeOH (9.7 l) and 70% Me_2CO (2.6 l); 15 ml frs) to yield 18 main fractions (indicated below with roman numbers). Each main fraction was further separated by Multi-layer Coil Countercurrent Chromatography (MLCCC), which was carried out with the solvent system EtOAc–*n*-PrOH–H_2O (140:8:80) on P.C. Inc. ITO Multi-layer Coil Separator–Extractor, flow rate 1.0 ml min^{-1}, using the upper-phase as mobile phase (these frs are indicated below with asterisks). All subfrs obtained were subjected to semi-prep. chromatography on reversed-phase C 18 (8 μm, 250 × 20 mm, Latek, Germany) under high pressure (HPLC) with solvents systems: MeOH–MeCN–H_2O (15:5:80; system S3) and different mixtures of MeOH–H_2O (system S4) at a flow rate of 10 ml min^{-1} (compounds were numbered according to their series of elution).

4′-O-Methyl-gallocatechin (**4**). Fr. IV (frs 144–180, 326 mg) was subjected to MLCCC (4 frs, 10 ml fr^{-1}). The subfraction * 2 (frs 18–34, 139 mg) was finally separated by HPLC (S3). The first substance obtained was gallic acid (R_t 10.2 min, 60.8 mg). The physical data were identical to those of an authentic sample. Compound **4** (R_t 26.5 min, *S*4, 50 mg) gave a prominent light brown spot at R_t 0.88 in TLC (S1). A portion (15 mg) of this compound was acetylated and purified by prep. TLC (S2) resulting in **4a** (10 mg). As free phenolic compound **4** gave: $[\alpha]_D^{20^\circ}$ + 28° (50% acetone, *c* 0.2) and EI-MS *m/z* (rel. int. %): 320 $[M]^+$ (14), 302 (6.3), 182 (52), 167 (55), 139 (100), 69 (39), 55 (48). The acetate **4a** gave the following data: ^{1}H NMR ($CDCl_3$, relative to $CHCl_3$ at δ7.26; 200 MHz): δ2.00 (OAc aliphatic, *s*), 2.25–2.36 (4 × phenolic OAc, all *s*), 2.65 (H-4_{ax}, *dd*, *J* = 7.7 and 16.1 Hz), 2.88 (H-4_{eq}, *dd*, *J* = 5.1 and 16.1 Hz), 3.80 (*O*-Me, *s*, 3H), 5.08 (H-2, *d*, *J* = 6.3 Hz), 5.20 (H-3, *ddd*, *J* = 6.3, 7.7 and 5.1 Hz), 6.59 (H-6, *d*, *J* = 2.2 Hz), 6.66 (H-8, *d*, *J* = 2.2 Hz), 6.97 (H-2′ and H-6′, *s*, 2H).

Gallocatechin (**1**), *epigallocatechin* (**2**) *and epigallocatechin 3-O-gallate* (**3**). Fr. V (frs 181–263; 1176 mg) was rechromatographed with MLCCC to give 5 subfrs. Sbfr. *3 (frs 51–60, 370 mg) was subjected to chromatography on HPLC (isocratic system S3), yielding compounds **1** (R_t 15.4 min, 104 mg) and **2** (R_t 20.4 min, 205 mg). A portion was acetylated to yield the acetates **1a** and **2a**. Their physical and spectroscopic data were identical to those of authentic samples. Compound **3** was obtained from Sephadex LH-20 fr. VIII (frs 481–629, 1307 mg) and subsequently rechromatographed with MLCCC (5 subfrs). Subfr. *1 (frs 1–14, 500 mg) was acetylated (50 mg) and purified by prep. TLC (system S2) to give **3a** (45 mg). Compound **3a** was identified as epigallocatechin 3-*O*-gallate by comparison of the physical data of its peracetate (^{1}H NMR, $[\alpha]_D^{20^\circ}$) with those of an authentic sample.

Epigallocatechin 3-O-(3,5-dimethyl)gallate (**5**) *and epigallocatechin 3-O-(3-methoxy-4-hydroxy)benzoate* (**6**). Fr. VII (frs 323–480; 2034 mg) was separated on MLCCC into six subfractions (10 ml frs). Subfraction *2 (frs 15–28; 868 mg) was again rechromatographed on Sephadex LH-20 column (50 × 3.5 cm; eluents: 50% MeOH (2 l), 75% MeOH (1 l); 15 ml fr.) to give seven frs. Fr. 5 (frs 242–275; 134 mg) was subjected to chromatography on HPLC (isocratic system S3) and gave four subfrs. Subfr. 2 (R_t 15.8 min, 28 mg) was acetylated and semipurified by prep. TLC (system S2) to give 12 mg. The acetylated mixture of substances was again rechromatographed on HPLC (normal-phase column, Lichrospher 100 CN, 5 μm, 250 × 4 mm, Merck; solvent system: *n*-hexane–EtOAc 55:45; flow rate 1.0 ml min^{-1}; detection: UV 278 nm) [9]. This system allowed a better separation of compounds **5a** (R_t 24.5 min; 6 mg) and **6a** (R_t 23.4 min; 4 mg). Compound **5a**: $[\alpha]_D^{20^\circ}$ −24.7° (acetone–H_2O 1:1, *c* 0.15). DCI-MS $[M + 18]^+$ *m/z* (rel. int. %) 756 (100), 742 (6), 684 (5). EI-MS *m/z* (rel. int. %): 738 $[M]^+$ (0.4), 696 (7.2), 498 (25), 414 (16), 372 (14), 330 (8), 287 (8), 223 (5), 181 (33), 139 (11), 97 (16), 55 (44), 43 (100). ^{1}H NMR ($CDCl_3$, 200 MHz): δ2.18–2.38 (6 × phenolic OAc, all *s*), 2.98–3.10 (H-4_{ax} and H-4_{eq}, *m*, 2H), 3.79 (2 × *O*-methyl, *s*, 6H), 5.23 (H-2, *brs*), 5.65 (H-3, *m*), 6.59 (H-6, *d*, *J* = 2.2 Hz), 6.74 (H-8, *d*, *J* = 2.2 Hz), 7.09 (H-2 and H-6; acyl group; *s*, 2H), 7.27 (H-2(B) and H-6(B), *s*, 2H). Compound **6a**: $[\alpha]_D^{20^\circ}$ −27° (acetone, *c* 0.1). DCI-MS $[M + 18]^+$ *m/z* (rel. int. %): 726 (100), 696 (12). EI-MS *m/z* (rel. int. %): 708 $[M]^+$ (6), 696 (46), 498 (10), 456 (34), 414 (26), 372 (20), 330 (9), 287 (10), 223 (4), 193 (10), 181 (25), 151 (42), 139 (8), 55 (11), 43 (100). ^{1}H NMR ($CDCl_3$, 200 MHz): δ2.15–246 (6 × phenolic OAc, all *s*), 2.98–3.12 (H-4_{ax} and H-4_{eq}, *m*, 2H), 3.82 (*O*-methyl, *s*, 3H), 5.22 (H-2, *brs*), 5.64 (H-3, *m*), 6.60 (H-6, *d*, *J* = 2.2 Hz), 6.74 (H-8, *d*, *J* = 2.2 Hz), 7.26 (H-2(B) and H-6(B), *s*, 2H), 7.02 (H-5; acyl group; *d*, *J* = 7.8 Hz), 7.44–7.50 (H-2 and H-6; acyl group; *m*, 2H).

Acid hydrolysis of **5a** *and* **6a**. 1 mg each were dissolved in 2 M HCl in EtOH (5 ml) and refluxed for 1 hr. The reaction mixt. was diluted with H_2O (10 ml) and subsequently extracted with EtOAc (2 × 15 ml). The organic phase was reduced in vol. and chromatographed on silica gel (TLC) in EtOAc–toluene (9:11), $CHCl_3$–HAc (9:1), Et_2O–petrol (1:1) and toluene–$EtOCO_2H$–HCO_2H (5:4:1). Syringic acid (from **5a**) and vanillic acid (from **6a**) were identified (TLC) by comparison with authentic samples.

Epigallocatechin-(4β → 8)-gallocatechin (**7**), *epigallocatechin-(4β → 8)-epigallocatechin* (**8**) *and epigallocatechin-(4β → 8)-epigallocatechin 3-O-gallate* (**9**). Fr. VI (frs 264–322; 479 mg) was subjected to MLCCC to yield seven subfractions. Subfr. *7 (frs 69–80; 69 mg) was purified by HPLC (isocratic system S3) to give four compounds. Compound 3 (R_t 6.2 min, 16 mg; R_t 0.32 in system S1 as free phenol) was acetylated and purified by prep. TLC (system S2, R_t 0.59) to give **7a** (8 mg). DCI-MS $[M + 18]^+$ *m/z* (rel. int. %): 1132 (43), 1090 (100), 1048 (24), 1012 (9). CD: $[\Theta]_{230}$ + 35000. The ^{1}H NMR data are identical with those published in [10].

Fr. VII (frs 323–480; 2034 mg) was subjected to MLCCC to yield six subfrs. Purification of subfr. *6 (frs 67–83; 209 mg) was carried out on HPLC (isocratic system S3) to give seven compounds. Compound 3, 30 mg, (R_t 7.1 min.; 68 mg) was acetylated and purified by prep. TLC (system S2; R_f 0.43) resulting in **8a** (25 mg). DCI-MS $[M + 18]^+$ *m/z* (rel. int. %): 1132 (100), 1090 (30), 1048 (11), 1012 (4), 969 (1). CD: $[\Theta]_{230} + 32000$. Physical data was identical to published data [11].

Fr. X (frs 701–934; 497 mg) was rechromatographed on MLCCC to give five subfractions (15 ml frs). Subfraction *5 (frs 58–79: 254 mg) was chromatographed by HPLC (isocratic system S4; 3:1) to give five substances. Substance 4, 35 mg, (R_t 11.3 min, 176 mg) was acetylated and purified by prep. TLC (system S2; R_f 0.32) consisting of **9a** (40 mg). DCI-MS $[M + 18]^+$ *m/z* (rel. int. %): 1368 (86), 1326 (100), 1283 (95), 1242 (54), 1200 (44), 1157 (26), 1115 (14), 948 (8), 905 (2.5), 314 (10). CD: $[\Theta]_{236} - 25000$, $[\Theta]_{280} - 15000$. The spectral data are identical with those recently published [11].

Epigallocatechin-(4β → 8)*-epigallocatechin 3-O-p-hydroxybenzoate* (**10**). A total of 1307 mg of fr. VIII (frs 481–629) were subjected to MLCCC to yield five subfrs. Subfr. *3 (frs 20–29, 173 mg) was rechromatographed on HPLC (isocratic system S4, 21:79) consisting of six compounds. A portion of the compound 4 (R_f 29 min, 77 mg) was peracetylated and purified by prep. TLC on silica gel (system S2; R_f 0.35) to give **10a** (20 mg). DCI-MS $[M + 18]^+$ *m/z* (rel. int. %): 1252 (100), 1210 (14), 1168 (6), 1130 (8), 1015 (14), 974 (3). CD: $[\Theta]_{243} - 24000$, $[\Theta]_{273} - 13000$. ^{1}H NMR (CDCl$_3$, 200 MHz): δ1.89–2.64 (OAc, *m*), 2.94–3.18 (H-4$_{ax}$ and H-4$_{eq}$ (F), *m*, 2H), 4.51 (H-4(C), *d*, *J* = 1.7 Hz), 4.66 (H-2(F), *br s*), 5.18 (H-3(C), *m*), 5.28 (H-3(F), *m*), 5.67 (H-2(C), *br s*), 6.17 (H-6(A), *d*, *J* = 2.2 Hz), 6.27 (H-8(A), *d*, *J* = 2.2 Hz). 6.67 (H-6 (D), *s*), 6.99 (H-2′ and H-6′ (E), *s*, 2H), 7.06 (H-3″ and H-5″ (*p*-hydroxybenzoyl), *d*, *J* = 8.8 Hz, 2H), 7.25 (H-2′ and H-6′ (B), *s*, 2H), 7.90 (H-2″ and H-6″ [*p*-hydroxybenzoyl], *d*, *J* = 8.8 Hz, 2H).

Epigallocatechin 3-O-gallate-(4β → 8)*-epigallocatechin 3-O-gallate* (**11**). Fr. XII (frs 1037–1100, 854 mg) was chromatographed on MLCCC resulting in six subfrs. Subfr. *3 (frs 25–38, 181 mg) was chromatographed on HPLC (gradient system S4; 25 → 30% MeOH at 32 min) yielding four compounds. Compound 4 (R_t 32.8 min, 51 mg) was acetylated and purified by prep. TLC (system S2, R_f 0.21) resulting in **11a** (19 mg). DCI-MS $[M + 18]^+$ *m/z* (rel. int. %): 1605 (64), 1562 (84), 1520 (100), 1478 (76), 1439 (52), 1397 (37), 1354 (37), 1326 (52), 1310 (50), 1266 (59), 1224 (62), 1182 (38), 1140 (20), 972 (8), 888 (9). CD: $[\Theta]_{225} - 60000$, $[\Theta]_{280} - 23000$. ^{1}H NMR (CDCl$_3$, 200 MHz): δ1.84–2.36 (OAc, *m*), 3.02–3.09 (H-4$_{ax}$ and H-4$_{eq}$ (F), *m*, 2H), 4.49 (H-4(C), *d*, *J* = 3.0 Hz), 4.76 (H-2(F), *br s*), 5.27 (H-3(F), *m*), 5.64 (H-2(F) and H-3(C), *m*, 2H), 6.31 (H-6 and H-8(A), *s*, 2H), 6.69 (H-6(D), *s*), 7.08 (H-2 and H-6(E), *s*, 2H), 7.22 (H-2 and H-6(B), *s*, 2H), 7.56 (H-2 and H-6 (galloyl at the 3- or 3′-*O* position), *s*, 2H), 7.75 (H-2 and H-6 (galloyl at the 3′- or 3-*O* position), *s*, 2H).

Epigallocatechin-(4β → 6)*-epigallocatechin* (**12**). Fr. XI (frs 935–1036, 1600 mg) was rechromatographed on MLCCC to yield five subfractions. Subfr. *5 (frs 65–88, 476 mg) was subjected to chromatography on HPLC (gradient system S4, 23–35% MeOH at 35 min.) to give six compounds. A sample of 15 mg of compound **5** (R_t 26.1 min, 76 mg) was acetylated and purified on prep. silica gel (system S2, R_f 0.43) yielding **12a**. DCI-MS $[M + 18]^+$ *m/z* (rel. int. %): 1132 (68), 1090 (100), 1048 (58), 1005 (14), 962 (5). CD: $[\Theta]_{240} + 135000$. ^{1}H NMR (CDCl$_3$, 200 MHz): δ1.15–2.47 (OAc, *m*), 2.86–3.02 (H-4$_{ax}$ and H-4$_{eq}$ (F), *m*, 2H), 5.15 (H-2(F), *br s*), 5.42–5.48 (H-3(F), *m*), 6.57–6.80 (H-6 and H-8(A) and H-8(D), *m*, 3H), 7.18 (H-2 and H-6(B) or (E), *s*, 2H), 7.23 (H-2 and H-6(E) or (B), *s*, 2H).

Gallocatechin-(4α → 8)*-epigallocatechin 3-O-gallate* (**13**). Fr. XIII (frs 1101–1180, 1088 mg) was subjected on MLCCC to give six subfractions. Subfr. *4 (frs 42–48, 164 mg) was rechromatographed on HPLC (gradient system S4, 24–27% MeOH at 20 min.) to obtain three compounds. A sample of 25 mg of compound **2** (R_t 13.2 min, 46 mg) was acetylated and purified by prep. TLC (system S2, R_f 0.22) to give **13a** (27 mg). DCI-MS $[M + 18]^+$ *m/z* (rel. int. %): 1368 (100), 1327 (20), 886 (4), 767 (4), 733 (8), 510 (7), 512 (7), 452 (16), 428 (37). CD: $[\Theta]_{220} - 142000$, $[\Theta]_{280} - 45000$. ^{1}H NMR (CDCl$_3$, 200 MHz): δ1.68–2.42 (OAc, *m*), 2.83 (H-4ax(F), *dd*, *J* = 18 Hz), 3.12 (H-4$_{eq}$(F), *dd*, *J* = 4.8 and 18 Hz), 4.64 (H-4(C), *d*, *J* = 10 Hz), 4.96 (H-2(C), *d*, *J* = 9.8 Hz), 5.20 (H-2(F), *br s*), 5.61–5.68 (H-3(C), *m*), 5.75 (H-3(F), *m*), 6.51 (H-6(A), *d*, *J* = 2.3 Hz), 6.59 (H-8(A), *d*, *J* = 2.3 Hz), 6.72 (H-6(D), *s*), 6.76 (H-2 and H-6(E), *s*, 2H), 7.03 (H-2 and H-6(B), *s*, 2H), 7.64 (H-2 and H-6 (galloyl), *s*, 2H).

Gallocatechin-(4α → 8)*-epigallocatechin 3-O-p-hydroxybenzoate* (**14**). Fr. XI (frs 935–1036; 1602 mg) was rechromatographed by MLCCC to obtain five subfrs. Subfr. *2 (frs 17–32, 253 mg) was purified by HPLC (gradient system S4, 28–30% MeOH at 15 min) to yield five compounds. A portion (18 mg) of compound 3 (R_t 14.2 min, 52 mg) was acetylated and purified by prep. TLC (system S2; 20 mg) consisting of **14a**. DCI-MS $[M + 18]^+$ *m/z* (rel. int. %): 1252 (100), 1210 (23), 1168 (2), 1015 (2). CD: $[\Theta]_{240} - 100000$, $[\Theta]_{280} - 25000$. ^{1}H NMR (CDCl$_3$, 200 MHz): δ1.67–2.40 (OAc, *m*), 2.90 (H-4$_{ax}$ (F), *dd*), 3.14 (H-4$_{eq}$ (F), *dd*, *J* = 4.8 and 18 Hz), 4.62 (H-4(C), *d*, *J* = 9.7 Hz), 4.92 (H-2(C), *d*, *J* = 10 Hz) , 5.18 (H-2(F), *br s*), 5.62 (H-3(F), *m*), 5.80 (H-3(C), *t*, Σ*J* = 19.7 Hz), 6.58 (H-6(A), *d*, *J* = 2.3 Hz), 6.67 (H-8(A), *d*, *J* = 2.3 Hz), 6.69 (H-6(D), *s*), 6.91 (H-2 and H-6(E), *s*, 2H), 6.98 (H-2 and H-6(B), *s*, 2H), 7.08 (H-3 and H-5(*p*-hydroxybenzoyl), *d*, *J* = 8.7 Hz, 2H), 7.79 (H-2 and H-6 (*p*-hydroxybenzoyl), *d*, *J* = 8.7 Hz, 2H).

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