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PROROBINETINIDINS FROM *STRYPHNODENDRON ADSTRINGENS*

JOÃO PALAZZO DE MELLO, FRANK PETEREIT and ADOLF NAHRSTEDT*

Institut für Pharmazeutische Biologie und Phytochemie, Westfälische Wilhelms-Universität, Hittorfstr. 56, D-48149 Münster, Germany

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Key Word Index—*Stryphnodendron adstringens*; Mimosaceae; stem bark; proanthocyanidins; prorobinetinidins.

Abstract—The natural occurrence of several new prorobinetinidins in the stem bark of *Stryphnodendron adstringens* has been demonstrated. These are robinetinidol-(4$\beta \rightarrow$8)-epigallocatechin, robinetinidol-(4$\alpha \rightarrow$8)-epigallocatechin, robinetinidol-(4$\beta \rightarrow$8)-epigallocatechin 3-*O*-gallate, robinetinidol-(4$\alpha \rightarrow$8)-epigallocatechin 3-*O*-gallate, robinetinidol-(4$\alpha \rightarrow$6)-gallocatechin and robinetinidol-(4$\alpha \rightarrow$6)-epigallocatechin, in addition to the tentatively characterized, robinetinidol [4$\beta \rightarrow$6(8)]-gallocatechin and robinetinidol-(4$\alpha \rightarrow$8)-gallocatechin. The structures were established on the basis of chemical and spectral evidence from their peracetate derivatives.

INTRODUCTION

The stem bark of *Stryphnodendron adstringens*, known to be rich in tannins [1], has been used by the native population of Brazil as a remedy for various diseases [2, 3]. The isolation and structures of flavan-3-ols and prodelphinidins from this species have been recently reported [4]. In continuation of our investigations on the polyflavanoid fraction to determine the pharmacologically active constituents from this source, we report here on the isolation and structure elucidation of additional dimeric prorobinetinidins (**1**–**8**).

RESULTS AND DISCUSSION

The ethyl acetate-soluble fraction obtained from the aqueous acetone extract of the air-dried stem bark was chromatographed on Sephadex LH-20 and fractions containing oligoflavanoids were further purified by multi-layer coil countercurrent chromatography (MLCCC) and HPLC on RP-18 to give compounds **1**–**8**. The identity of all biflavanoid prorobinetinidins (**1**–**8**) discussed below was established by physical properties [^{1}H NMR, circular dichroism (CD), DCI-mass spectrometry] of the corresponding peracetate derivatives. Compared to the ^{1}H NMR ($CDCl_3$) of known procyanidin and prodelphinidin peracetates, similarities are obvious, except for the A-ring region and the diagnostic chemical shifts of heterocyclic C-ring protons. It is noteworthy that most of the ^{1}H NMR chemical shift criteria derived from structural elucidation of peracetylated dimeric procyanidins and prodelphinidins, useful for distinguishing (4$\rightarrow$8) and (4$\rightarrow$6) interflavanyl linkages [5–7], are also of diagnostic value for the analogous prorobinetinidin peracetates.

Compound **1** exhibited a $[M + 18]^+$ peak at *m/z* 1074 in the DCI-mass spectrum of the peracetate (**1a**), indicating a biflavanoid proanthocyanidin with different hydroxylated flavan-3-ol units. The aromatic substitution pattern and, hence, the 5-deoxyproanthocyanidin character, were evident from an AMX spin system (δ 6.29–6.86), a residual D-ring proton at δ 6.66 and two two-proton singlets at δ 7.15 and 6.80 in the ^{1}H NMR of **1a**. The latter signals were assigned to the equivalent B- and E-ring protons, respectively, with the aid of a ^{1}H–^{1}H COSY experiment with the 2-H(C) and 2-H(F) resonances as reference signals. The coupling constants of the C-ring protons ($J_{2,3}$ 7.7 Hz; $J_{3,4}$ = 5.4 Hz) confirm the 2,3-*trans*-3,4-*cis* relative configuration, whereas the 2,3-*cis* configuration of the 'lower' flavan-3-ol unit is evident from the small coupling constants of the heterocyclic protons ($J_{2,3(F)} < 2.0$ Hz). In the case of methoxyacetylated 5-deoxyproanthocyanidins, the chemical shift and splitting pattern of the proton H-3(C) are useful for the differentiation of dimers with 2,3-*trans*-3,4-*cis* and 2,3-*trans*-3,4-*trans* configuration [8]. The chemical shift and the splitting pattern of proton H-3(C) at δ 5.33 in **1a** are similar to those of methoxyacetylated biflavanoids of corresponding configuration and suggest a 2,3-*trans*-3,4-*cis* stereochemistry. The 2*R*,3*S*,4*R* absolute configuration was determined from the high-amplitude positive Cotton effect in the 210–240 nm region of the CD spectrum of **1a** [9, 10]. Evidence for the (4$\rightarrow$8) interflavanyl linkage stems from the chemical shift of H-6(A) (δ 6.47), H-8(A) (δ 6.29) [5] and H-2(F) (δ 4.45) [6], in conjunction with the clear dominance of one rotamer

*Author to whom correspondence should be addressed.

(see below) [11]. Confirmation of the proposed structure was based on acid hydrolysis of **1**, which liberated robinetinidin and epigallocatechin (see Experimental). Thus, **1** was identified as robinetinidol-(4$\beta \rightarrow$8)-epigallocatechin.

The peracetate derivative (**2a**) of **2** produced a $[M + 18]^+$ peak at *m/z* 1074 in the DCI-mass spectrum. The ^1H NMR spectrum of **2a** had the characteristic spin system of an all-*trans* dimeric prorobinetinidin, as evidenced by the coupling constants of the heterocyclic protons ($J_{2,3(C)} = 9.6$ Hz; $J_{3,4(C)} = 9.8$ Hz; $J_{2,3(F)} = 8.6$ Hz), an aromatic AMX spin system and two two-proton singlets at δ 6.97 and 6.65, indicating the presence of pyrogallol-type B- and E-rings, respectively. The determination of the 'upper' 5-deoxyflavan 3-ol unit was facilitated by the long-range coupling between H-5(A) (δ 6.89) and H-4(C) (δ 4.51) ('W'-coupling) from a ^1H–^1H COSY experiment. The dominance of one rotamer [11], the chemical shift of H-3(C) (δ 5.79; *t*, 19.6 Hz) [8], the chemical shift of the B-ring protons [7] and the high-amplitude negative Cotton effect in the 220–240 nm region of the CD spectrum of **2a**, all confirmed a (4$\alpha \rightarrow$8) linkage and, thus, a 4*S* absolute configuration. Consequently, **2** was characterized as robinetinidol-(4$\alpha \rightarrow$8)-gallocatechin, previously reported as a prominent metabolite from *Acacia mearnsii* [8, 12–14].

Compound **3** showed a parent ion at *m/z* 1074 $[M + 18]^+$ in the DCI-mass spectrum of the corresponding peracetate (**3a**), again suggesting a dimeric prorobinetinidin. Determination of the 'upper' robinetinidol unit was made possible by the presence of a long-range coupling of H-5(A) with H-4(C) in the ^1H–^1H COSY spectrum. The 2,3-*trans*, 3,4-*trans*(C); 2,3-*cis* (F) relative configuration was evident from coupling constants ($J_{2,3(C)} = 9.8$ Hz; $J_{3,4(C)} = 9.9$ Hz; $J_{2,3(F)} < 2.0$ Hz) compatible with such stereochemistry. The aromatic AMX system and two two-proton singlets at δ 6.98 and 6.68 for the B- and E-ring protons, respectively, confirm the aromatic substitution pattern, while the chemical shift of H-3(C) at δ 5.87 (*t*, $\sum J =$ 20.0 Hz) strongly favours the 2,3-*trans*, 3,4-*trans* stereochemistry [8]. Evidence for the (4$\rightarrow$8) interflavanyl linkage was based on the chemical shift of B-ring protons [7] and the dominance of one rotamer [11]. This assignment was supported by a negative Cotton effect in the 210–240 nm region of the CD spectrum of **3a**, indicating a 4α-flavanyl substituent and, thus, a 4*S* absolute configuration. Accordingly, **3** was characterized as robinetinidol-(4$\alpha \rightarrow$8)-epigallocatechin.

The prorobinetinidin biflavanoid, robinetinidol-[4$\beta \rightarrow$6(8)]-gallocatechin (**4**), was again described by the physical data for the corresponding peracetate (**4a**). The coupling constants of the heterocyclic protons ($J_{2,3(C)} = 6.5$ Hz; $J_{3,4(C)} = 5.2$ Hz; $J_{2,3(F)} = 7.6$ Hz) and the upfield position of H-3(C) at δ 5.34 [8] in the ^1H NMR spectrum correspond to a 2,3-*trans*, 3,4-*cis* (C); 2,3-*trans* (F) configuration. The 4*R* absolute configuration was supported by the positive Cotton effect of **4a** at 210–240 nm. In some previous studies, (4$\rightarrow$8) and (4$\rightarrow$6) interflavanyl linkage have been distinguished by the dominance of a single rotamer (4$\rightarrow$8) or the presence of a rotameric population of *ca* 1:1 (4$\rightarrow$6), respectively [11]. However, in the case of **4a**, most resonances consist of sharp. single peaks, suggesting the presence of only one rotameric form. This single preferred rotamer could be explained by the relatively high energy barrier to rotation about the interflavanyl linkage of 3,4-*cis*-biflavanoids relative to those with 3,4-*trans* configuration [15]. Thus, rotamer proportion and the line-broadening of resonances alone are not adequate to distinguish the point of linkage of biflavanoids with a 3,4-*cis* configuration. Based on this information, the chemical shifts of H-6(A) (δ 6.59), H-8(A) (δ 6.77), H-2(F) (δ 5.03) and the B- and E-ring protons at δ 7.13 and 7.15, respectively, correlate better with a (4$\alpha \rightarrow$8) or (4$\beta \rightarrow$6), rather than a (4$\beta \rightarrow$8) linkage [5–7]. A 4α (4*S*) configuration and a (4$\alpha \rightarrow$8)-linkage could be excluded by the above arguments (coupling constants and chemical shifts of C-ring protons, and CD spectrum) and the identification of **2**. However, the proposed (4$\beta \rightarrow$6) linkage for **4** is speculative and requires confirmation via synthesis.

Compound **5** had a $[M + 18]^+$ at *m/z* 1310 in the DCI-mass spectrum of the peracetate (**5a**), indicating a monogalloylated dimeric prorobinetinidin. The 3′-*O*-galloyl ester of **1** was identified by comparison of the physical data for the peracetate (**5a**) with those of the closely related peracetate derivative (**1a**). The structural similarity of **5a** and **1a** became evident from the ^1H NMR comparison, except for an additional two-proton singlet at δ 7.70 and a downfield shift (Δ 0.13 ppm) of H-3(F) (δ 5.28) in **5a**, indicative of the presence of a galloyl moiety at the C-3(F) hydroxyl. The relative 2,3-*trans*, 3,4-*cis*(C); 2,3-*cis* (F) stereochemistry was evident from coupling constants of the heterocyclic protons ($J_{2,3(C)} = 9.0$ Hz; $J_{3,4(C)} = 6.1$ Hz; $J_{2,3(F)} <$ 2 Hz) and the chemical shift of the H-3(C) at δ 5.27 [8]. Prorobinetinidins with this configuration (**5a** and **1a**) are characterized by the chemical shift reversal of H-6(A) (δ 6.45) and H-8(A) (δ 6.25) relative to those with a 2,3-*trans*-3,4-*cis* (C); 2,3-*trans* (F) stereochemistry (**4a**) and the absence of a cross-peak ('W'-coupling) between the H-5(A) and H-4(C) in their ^1H–^1H COSY spectra. Evidence for the (4$\rightarrow$8) interflavanyl linkage comes from the dominance of one conformer [11], the upfield shift of H-2(F) (δ 4.44) and the chemical shifts of A-ring protons [5, 6]. The negative Cotton effect in the 210–240 nm region of the CD spectrum of **5a** is in agreement with the observed Cotton-effect reversal of 3′-*O*-acylated dimeric prodelphinidin-peracetates with a 4*R* stereochemistry [16]. The proposed structure was supported by acid hydrolysis of **5**, which yielded epigallocatechin 3-*O*-gallate, identified by TLC with an authentic sample as the 'terminal' flavan-3-ol unit. Thus, **5** was identified as robinetinidol-(4$\beta \rightarrow$8)-epigallocatechin 3-*O*-gallate.

The biflavanoid robinetinidol-(4$\alpha \rightarrow$6)-gallocatechin (**6**), was characterized as the peracetate (**6a**) in a similar

manner. The ^{1}H NMR displayed a characteristic AMX spin system, two two-proton singlets at δ 7.24 and 7.18, attributable to the equivalent B- and E-ring protons, respectively. The all-*trans* configuration was again indicated by the characteristic spin pattern of the heterocyclic protons ($J_{2,3(C)} = 9.5$ Hz; $J_{3,4(C)} = 10$ Hz; $J_{2,3(F)} = 9.6$ Hz) and the chemical shift of H-3(C) at δ 5.72 ($\Sigma J = 19.5$ Hz) [8]. Two sets of signals in the ratio *ca* 1:1 suggest the presence of a (4→6) linked dimer [11]. The 4α-flavanyl linkage and, thus, the 4*S* configuration was supported by the strong negative Cotton effect in the diagnostic 210–240 nm region of the CD spectrum. Accordingly, **6** was identified as robinetinidol-(4α→6)-gallocatechin. To the best of our knowledge, the natural occurrence of **6** is described here for the first time.

Comparison of the ^{1}H NMR spectral data for the acetate derivative of **7** (**7a**) with the corresponding derivative of procyanidin B_8 [6] reveals their close structural resemblance. The aromatic region of **7a** includes an AMX spin system and two two-proton singlets, characteristic of a 5-deoxyflavan-3-ol unit. Duplication of signals due to dynamic rotational isomerism strongly favours the (4→6) interflavanyl linkage [11]. The 2,3-*trans*, 3,4-*trans* (C); 2,3-*cis* (F) stereochemistry was determined by the heterocyclic coupling constants ($J_{2,3(C)} = 9.9$ Hz; $J_{3,4(C)} = 9.7$ Hz; $J_{2,3(C)} < 2$ Hz) and the chemical shift of H-3(C) at δ 5.67 [8]. Further evidence for the 4α-flavanyl linkage and, thus, the 4*S* absolute configuration was based on the negative Cotton effect in the CD spectrum of the compound at 210–240 nm. The proposed structure was supported by the acid cleavage products of **7** and the DCI-mass spectral data for **7a** (see Experimental). Thus, **7** was identified as robinetinidol-(4α→6)-epigallocatechin.

Identification of robinetinidol-(4α→8)-epigallocatechin 3-*O*-gallate (**8**) was effected by comparison of the ^{1}H NMR spectral data for **8a** with those for **3a**. Apart from an additional two-proton singlet indicative of the equivalent 2- and 6-protons of a galloyl moiety at δ 7.43 in **8a**, their spin patterns were superimposable. The point of attachment of the galloyl group was ascertained by the significant downfield shift (Δ 0.34 ppm) of the H-3(F) proton (δ 5.65) in comparison to that of the parent compound (**3a**) at δ 5.31. The 2,3-*trans*, 3,4-*trans* (C); 2,3-*cis* (F) relative stereochemistry was suggested by the coupling constants of the heterocyclic protons ($J_{2,3(C)} = 9.8$ Hz; $J_{3,4(C)} = 10$ Hz; $J_{2,3(F)} < 2$ Hz) and the chemical shift of H-3(C) at δ 5.85 ($\Sigma J = 20$ Hz) [8]. The chemical shift of the B- and E-ring [7] protons at δ 7.02 and 6.63, respectively, the dominance of one rotamer [11] and the negative Cotton effect in the CD spectrum of **8a** confirmed the (4α→8) interflavanyl linkage and, thus, the 4*S* stereochemistry. The proposed structure was supported by the DCI-mass spectrum of **8a**, which had a $[M + 18]^+$ at *m/z* 1310, and by the identification (TLC) of epigallocatechin 3-*O*-gallate after acid hydrolysis.

In conclusion, investigation of the ethyl acetate-soluble fraction of an acetone–H_2O extract from the stem bark of *S. adstringens* has led to the isolation and characterization of several prorobinetinidins (**1**–**8**), in addition to a series of flavan 3-ols and prodelphinidins [4]. In contrast to known prorobinetinidin dimers with catechin or gallocatechin as 'terminal' units, counterparts with a 2,3-*cis* configuration (**1**, **3**, **5**, **7** and **8**) from this class of polyflavanoids have not been demonstrated previously. Identification of **1** and **5** not only extends the series of naturally occurring 3,4-*cis*-prorobinetinidins, but also introduces the first 3,4-*cis*-prorobinetinidins associated with epigallocatechin and 3′-*O*-acylated epigallocatechin. Future investigation of extracts of this species includes elucidation of the presumed trimeric and oligomeric prorobinetinidins that accompany the dimers described in this report.

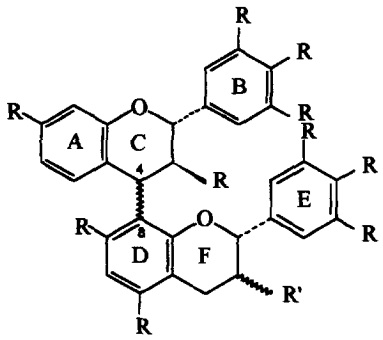
EXPERIMENTAL

General. NMR were recorded in $CDCl_3$ at ambient temp. with TMS as int. standard. CD data were obtained in MeOH. DCI spectra were obtained with NH_3 as reactant gas in the positive-ion mode. Compounds were visualized by spraying with vanillin–HCl reagent and 1% ethanolic $FeCl_3$ soln. Analyt. TLC was carried out on precoated aluminium sheets (Kieselgel 60 F_{254}) with EtOAc–HCO_2H–H_2O (18:1:1; system S1). Prep. TLC was performed on silica gel plates (Kieselgel 60 F_{254}, 0.5 mm) using toluene–Me_2CO (7:3; system S2). Acetylations were performed in pyridine–Ac_2O (1:1) at ambient temp.

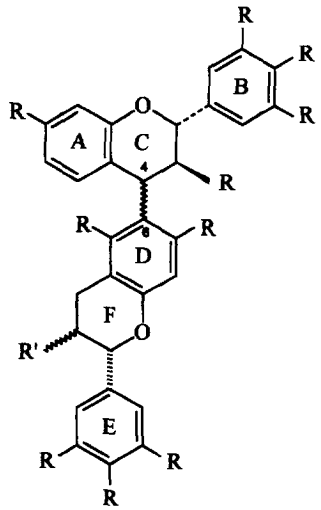
Conversion of prorobinetinidins into anthocyanidins. The prorobinetinidin (*ca* 1 mg) was refluxed with 5% HCl in EtOH for 1 hr. The reaction mixt. was subsequently chromatographed on cellulose (Cellulose F, 0.2 mm, Merck) using HCO_2H–HCl–H_2O (10:1:3). Due to the lack of an authentic sample of robinetinidin, the anthocyanidin liberated from **2** served as ref. substance. For an increase of the colour yield in the anthocyanidin reaction, the method described in refs [17, 18] was used. Each prorobinetinidin sample (1 mg) was dissolved in 0.2 ml *n*-BuOH–25% HCl (19:1). After addition of 5 μl of 2% soln of $NH_4Fe(SO_4)_2.12H_2O$ in 2 N HCl, the test tube was placed in a boiling-water bath for 1 hr.

Identification of 'lower' 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. [19] liberated the respective flavan 3-ol unit, which was detected by TLC on cellulose in H_2O–dioxane (10:1) or on silica gel in systems 1 using ref. substances available in our institute. Hydrolysis of epigallocatechin 3-*O*-gallate did not occur under these conditions [4].

Plant material. Stem bark of *S. adstringens* (Martius) Coville was collected in the Reserva de Cerrado-FAP-ESP (State of São Paulo, Brazil) and identified as discussed elsewhere [4]; a voucher specimen is deposited in the Herbarium of our institute (PBMS 73).



	R	R'	4 ⟷ 8
1	OH	OH	▬
1a	OAc	OAc	▬
2	OH	OH	·····
2a	OAc	OAc	·····
3	OH	OH	·····
3a	OAc	OAc	·····
5	OH	*O*-galloyl	▬
5a	OAc	*O*-galloyl-peracetate	▬
8	OH	*O*-galloyl	·····
8a	OAc	*O*-galloyl-peracetate	·····



	R	R'	4 ⟷ 6	
4	OH	OH	▬	*
4a	OAc	OAc	▬	*
6	OH	OH	·····	
6a	OAc	OAc	·····	
7	OH	OH	·····	
7a	OAc	OAc	·····	

*Mode of linkage is preliminary

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 red. pres. 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 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 afford 18 main frs (indicated below with roman numbers). Each main fr. was further sepd by MLCCC, which was carried out with the solvent system EtOAc-*n*-PrOH–H_2O (35:2:2) at 1 ml min^{-1}, using the upper layer as mobile phase (these frs are indicated below with #). All subfrs obtained were subjected to semi-prep. chromatography on reverse-phase C18, (8 μm, 250 × 20 mm, Latek, Germany) under high pressure (HPLC) with the solvent systems MeOH–MeCN–H_2O (3:1:16; system S3) and different mixts of MeOH–H_2O (system S4) at 10 ml min^{-1} (compounds are numbered according to their order of elution).

Robinetinidol-(4β→8)-epigallocatechin (**1**). Fr VI (frs 264–322; 479 mg) was subjected to MLCCC to give 7 subfrs. Subfr #6 (frs 58–68, 38 mg) was finally sepd by HPLC (system S3) to give 4 compounds. A portion (10 mg) of compound 1 (R_t 19 min.; 12.6 mg) was acetylated and purified by prep. TLC (S2; R_f 0.56) to give **1a** (13 mg). DCI-MS *m/z* (rel. int. %): 1074 (100) $[M + 18]^+$, 1031 (60), 989 (5), 947 (1), 823 (2). $CD[\Theta]_{235} = +28000$, $[\Theta]_{275} = -4000$. ^1H NMR ($CDCl_3$, 200 MHz): δ 1.85–2.30 (OAc, *m*), 2.89–2.98 [H-4ax, H-4eq(F), *m*, 2H], 4.45 [H-2(F), *s*], 4.49 [H-4(C), *d*, $J = 5.4$ Hz], 5.33 [H-3(C), H-3(F), *m*, 2H), 5.50 [H-2(C), *d*, $J = 7.7$ Hz], 6.29 [H-8(A), *d*, $J = 2.3$ Hz], 6.47 [H-6(A), *dd*, $J = 2.3$, 8.3 Hz], 6.66 [H-6(D), *s*], 6.80 [H-2′, H-6′(E), *s*, 2H], 6.86 [H-5(A), *d*, $J = 8.3$ Hz], 7.15 [H-2′,H-6′(B), *s*, 2H].

Robinetinidol-(4α→8)-gallocatechin (**2**). Fr. VII (frs 323–480; 2034 mg) was subjected to MLCCC to give 6 subfrs. Subfr. #5 (frs 51–66; 116 mg) was subjected to HPLC (S3) to afford 7 compounds. A

portion of compound 3 (R_t 19.6 min.; 79 mg) was acetylated and purified by prep. TLC (system S2; R_f 0.55) resulting in **2a** (27 mg). DCI-MS *m/z* (rel. int. %): 1074 (57), $[M+18]^+$, 1032 (100), 990 (77), 974 (32), 948 (44), 932 (28), 914 (11), 906 (14). CD:$[\Theta]_{235} = -19000$, $[\Theta]_{280} = -12000$. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.65–2.35 (OAc, *m*), 266 [H-4ax(F), *dd*, $J = 8.2$, 16.7 Hz], 2.99 [H-4eq(F), *dd*, $J =$ 5.9, 16.7 Hz], 4.51 [H-4(C), *d*, $J = 9.8$ Hz], 4.83 [H-3(F), *m*], 4.90 [H-2(C), *d*, $J = 9.6$ Hz], 5.04 [H-2(F), *d*, $J = 8.6$ Hz], 5.79 [H-3(C), *t*, $\Sigma J = 19.6$ Hz], 6.65 [H-2′ and H-6′(E); H-6 and H-8(A), H-6(D), *m*, 5H], 6.89 [H-5(A), *d*, $J = 8$ Hz), 6.97 [H-2′, H-6′(B), *s*, 2H].

Robinetinidol-(4$\alpha \rightarrow$8)*-epigallocatechin* (**3**). Fr VIII (frs 481–629, 1307 mg) was subjected to MLCCC to afford 5 subfrs. Subfr. #5 (frs 50–72, 173 mg) was submitted to final purification by HPLC (S4; 21:79) to afford 6 compounds. A portion (25 mg) of compound 5 (R_t 25.3 min.; 56 mg) was peracetylated and purified by prep. TLC (system S2, R_f 0.36) to give 22 mg **3a**. DCI-MS *m/z* (rel. int. %): 1074 (100) $[M+18]^+$, 1033 (46). CD: $[\Theta]_{235} = -14000$, $[\Theta]_{280} = -13000$. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.64–2.38 (OAc, *m*), 2.83 [H-4ax(F), *dd*], 3.01 [H-4eq(F), *dd*], 4.57 [H-4(C), *d*, $J = 9.9$ Hz], 4.98 [H-2(C), *d*, $J = 9.8$ Hz], 5.11 [H-2(F), *br s*], 5.32 [H-3(F), *m*], 5.87 [H-3(C), *t*, $\Sigma J =$ 20 Hz], 6.54–6.65 [H-6(A), H-8(A), H-6(D), *m*, 3H], 6.68 [H-2′, H-6′(E), *s*, 2H], 6.91 [H-5(A), *d*, $J =$ 7.5 Hz], 6.98 [H-2′, H-6′(B), *s*, 2H].

Robinetinidol-[4$\beta \rightarrow$6(8)]*-gallocatechin* (**4**) *and robinetinidol-*(4$\beta \rightarrow$8)*-epigallocatechin* 3*-O-gallate* (**5**). Fr. XI (frs 935–1036; 1602 mg) was subjected to MLCCC resulting in various subfrs. Subfr. #2 (frs 17–32); 253 mg) was subjected to HPLC (system S4; 28–30% MeOH at 15 min.) to afford 5 compounds. Portions of compounds 2 (10 mg) and 4 (12 mg) [R_t 7.95 min. and 15.31 min. (21 and 25 mg), respectively] were acetylated and purified by prep. TLC (system S2) to give 12 mg **4a** (R_t 0.44) and 11 mg **5a** (R_f 0.30).

Compound **4a**. DCI-MS *m/z* (rel. int. %): 1074 (100) $[M+18]^+$, 1032 (29), 1014 (6), 990 (4) CD: $[\Theta]_{235} =$ +140000. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.75–2.38 (OAc, *m*), 2.55 [H-4ax(F), *dd*, $J = 7.5$, 16 Hz], 3.02 [H-4eq(F), *dd*], 4.30 [H-4(C), *d*, $J = 5.2$ Hz], 5.03 [H-2(F), *d*, $J = 7.6$ Hz], 5.17 [H-3(F), *ddd*, $J = 5.7$, 7.5, 7.6 Hz], 5.34 [H-3(C), *dd*], 5.44 [H-2(C), *d*, $J =$ 6.5 Hz], 6.59 [H-6(A), *dd*, $J = 2.3$, 8.2 Hz], 6.65 [H-6(D), *s*], 6.77 [H-8(A), *d*, $J = 2.3$ Hz], 6.92 [H-5(A), *d*, $J = 8.2$ Hz], 7.13 [H-2′, H-6′(B), *s*, 2H], 7.15 [H-2′, H-6′(E), *s*, 2H].

Compound **5a**. DCI-MS *m/z* (rel. int. %): 1310 (100) $[M+18]^+$, 1268 (94), 1226 (58), 1209 (15), 1184 (17), 1142 (4), 1016 (5). CD: $[\Theta]_{228} = -21000$, $[\Theta]_{280} = -16000$. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.67–2.38 (OAc, *m*), 2.98–3.16 [H-4ax, H-4eq(F), *m*, 2H], 4.44 [H-2(F), *br s*], 4.51 [H-4(C), *d*, $J = 6.1$ Hz], 5.27 [H-3(C), H-3(F), *m*, 2H], 5.44 [H-2(C), *d*, $J =$ 9 Hz], 6.25 [H-8(A), *d*, $J = 2.2$ Hz], 6.45 [H-6(A), *dd*, $J = 2.2$, 8.5 Hz], 6.71 [H-6(D), *s*], 6.82 [H-5(A), *d*, $J = 8.5$ Hz], 7.01 [H-2′, H-6′(E), *s*, 2H], 7.21 [H-2′, H-6′(B), *s*, 2H], 7.70 [H-2, H-6(galloyl), *s*, 2H].

Robinetinidol-(4$\alpha \rightarrow$6)*-gallocatechin* (**6**) *and robinetinidol-*(4$\alpha \rightarrow$6)*-epigallocatechin* (**7**). Subfr. #4 (see isolation procedure for **4** and **5**; frs 46–64; 253 mg) was submitted to HPLC (S4; 25–30% MeOH at 25 min) to give 5 compounds. A portion of compound 3 (25 mg) (R_t 16.63 min.; 44 mg) and compound 5 (15 mg) (R_t 28.2 min.; 38 mg) was acetylated and purified by prep. TLC (system S2) to afford 21 mg (R_f 0.45) **6a** and 9 mg (R_t 0.41) **7a**.

Compound **6a**. DCI-MS *m/z* (rel. int. %): 1074 (100) [M + 18], 1032 (56), 1014 (31), 989 (52), 972 (21), 948 (22), 823 (13), 781 (14), 739 (5), 721 (7). CD: $[\Theta]_{235} = -22000$, $[\Theta]_{283} = -13000$. ^{1}H NMR ($CDCl_3$, 200 MHz; duplication due to dynamic rotational isomerism): δ 1.76–2.40 (OAc, *m*), 2.53 [H-4ax(F), *dd*, $J = 7.7$, 16.5 Hz], 2.97 [H-4eq(F), *dd*, $J = 5.3$, 16.5 Hz], 4.47 [H-4(C), *d*, $J = 9.9$ Hz], 4.54 [H-4(C), *d*, $J = 9.9$ Hz], 4.86–4.96 [2x H-2(C), 2x H-2(F), *m*, 4H], 5.01–5.06 [2x H-3(F), *m*], 5.72 [2x H-3(C), *t*, $\Sigma J =$ 19.5 Hz], 6.56–6.68 [2x H-6, H-8(A), 2x H-8(D), *m*, 6H], 6.87 [H-5(A), *d*, $J = 8.7$ Hz], 7.17 [H-2′, H-6′(E), *s*, 2H], 7.18 [H-2′, H-6′(E), *s*, 2H], 7.24 [2x H-2′, H-6′(B), *s*, 2H].

Compound **7a**. DCI-MS *m/z* (rel. int. %): 1074 (100) $[M+18]^+$, 1032 (8), 954 (2), 923 (4). CD: $[\Theta]_{235} =$ −33000, $[\Theta]_{280} = -9000$. ^{1}H NMR ($CDCl_3$, 200 MHz; duplication due to rotational isomerism): δ 1.61–2.36 (OAc, *m*), 2.75–2.92 [2x H-4ax, H-4eq(F), *m*, 4H], 4.56 [H-4(C), *d*, $J = 9.7$ Hz], 4.93 [H-2(C), *d*, $J =$ 9.9 Hz], 4.95 [H-2(C), *d*, $J = 9.9$ Hz], 5.12 [2x H-2(F), *d*, $J = 3.2$ Hz], 5.31 [H-3(F), *m*], 5.35 [H-3(F), *m*], 5.67 [H-3(C), *t*, $\Sigma J = 19.5$ Hz], 6.56–6.71 (2x H-6, H-8(A), 2x H-8(D), *m*, 6H], 6.86 [H-5(A), *d*, $J =$ 9.2 Hz], 7.21 [H-2′, H-6′ (E), *s*, 2H], 7.22 [H-2′, H-6′(E), *s*, 2H], 7.24 [2x H-2′, H-6′(B), *s*, 4H].

Robinetinidol-(4$\alpha \rightarrow$8)*-epigallocatechin* 3*-O-gallate* (**8**). Fr. XII (frs 1037–1100, 854 mg) was subjected to MLCCC to give 6 subfrns. Subfr. #2 (155 mg) (frs 16–24) was purified by HPLC (system S4; 25–30% MeOH at 32 min.) to afford 5 compounds. Compound 4 (26 mg) (R_t 37.5 min.; 57 mg) was peracetylated and purified by prep. TLC (system S2, R_f 0.25) to give 25 mg **8a**. DCI-MS *m/z* (rel. int. %): 1310 (100) $[M+18]^+$, 1268 (32), 1226 (14), 1184 (1), 1147 (1), 1014 (1). CD: $[\Theta]_{235} = -65000$, $[\Theta]_{280} = -35000$. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.65–2.39 (OAc, *m*), 2.93 [H-4ax(F), *dd*, $J = 2$ Hz, 17 Hz), 3.07 [H-4eq (F), *dd*, $J = 4$ Hz, 17 Hz], 4.60 [H-4(C), *d*, $J = 10$ Hz], 5.01 [H-2(C), *d*, $J = 9.8$ Hz], 5.26 [H-2(F), *br s*), 5.66 [H-3(F), *m*], 5.85 [H-3(C), *t*, $\Sigma J = 20$ Hz], 6.63–6.71 [H-6 amd H-8(A); H-2′ and H-6′(E), *m*, 4H], 6.75 [H-6(D), *s*], 6.95 [H-5(A), *d*], 7.02 [H-2′, H-6′(B), *br s*, 2H], 7.43 [H-2, H-6(galloyl), *s*, 2H].

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REFERENCES

1. *Farmacopeia Brasileira* (1959), p. 126.
2. Siqueira, J. C. de (1982) *Spectrum. J. Bras. Ci.* **2**, 41.
3. Santos, C. A., Torres, K. R. and Leonart, R. (1987) *Plantas Medicinais*, Curitiba, p. 39.
4. Palazzo de Mello, J., Petereit, F. and Nahrstedt, A. (1995) *Phytochemistry*, **41**, 807.
5. Hemingway, R. W., Foo, L. J. and Porter, L. J. (1982) *J. Chem. Soc., Perkin Trans. 1* 1209.
6. Kolodziej, H. (1992) in *Plant Polyphenols, Synthesis, Properties, Significance* (Hemingway, R. W. and Laks, P. E., eds), p. 295. Plenum Press, New York.
7. Danne, A., Petereit, F. and Nahrstedt, A. (1993) *Phytochemistry* **34**, 1129.
8. Botha, J. J., Ferreira, D. and Roux, D. G. (1981) *J. Chem. Soc., Perkin Trans. 1* 1235.
9. Barrett, M. W., Klyne, W., Scopes, P. M., Fletcher, A. C., Porter, L. J. and Haslam, E. (1979) *J. Chem. Soc., Perkin Trans. 1* 2375.
10. Botha, J. J., Young, D. A., Ferreira, D. and Roux, D. G. (1981) *J. Chem. Soc., Perkin Trans. 1* 1213.
11. Fletcher, A. C., Porter, L. J., Haslam, E. and Gupta, R. K. (1977) *J. Chem. Soc., Perkin Trans. 1*, 1628.
12. Drewes, S. E., Roux, D. G., Saayman, H. M., Feeney, J. and Eggers, S. H. (1966) *J. Chem. Soc., Chem. Commun.* 370.
13. Drewes, S. E., Roux, D. G., Saayman, H. M., Eggers, S. H. and Feeney, J. (1967) *J. Chem. Soc. (C)* 1302.
14. Botha, J. J., Ferreira, D. and Roux, D. G. (1978) *J. Chem. Soc., Chem. Commun.* 700.
15. Kolodziej, H. (1985) *Phytochemistry* **24**, 2460.
16. Danne, A., Petereit, F. and Nahrstedt, A. (1994) *Phytochemistry* **37**, 533.
17. Porter, L. J., Hrstich, L. N. and Chan, B. G. (1986) *Phytochemistry* **25**, 223.
18. Pallenbach, E. (1992) Ph.D. thesis, University of Freiburg.
19. Thompson, R. S., Jaques, D., Haslam, E. and Tanner, R. J. N. (1972) *J. Chem. Soc., Perkin Trans. 1* 1387.