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PROANTHOCYANIDINS WITH (+)-EPICATECHIN UNITS FROM *BYRSONIMA CRASSIFOLIA* BARK

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Key Word Index—*Byrsonima crassifolia*; Malpighiaceae; bark; proanthocyanidins; tannins; 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin; 3-*O*-galloyl-(−)-epicatechin-[4β → 8]-3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin; 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-3-*O*-galloyl-(+)-epicatechin; 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin; (+)-epicatechin-[4α → 8]-3-*O*-galloyl-(+)-epicatechin; (+)-epicatechin-[4α → 6]-(+)-epicatechin; 3-*O*-galloyl-(+)-epicatechin.

Abstract—Two new procyanidin trimers (3-*O*-galloyl-(+)-epicatechin-[4α → 8]-3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin and 3-*O*-galloyl-(−)-epicatechin-[4β → 8]-3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin), together with four new dimers (3-*O*-galloyl-(+)-epicatechin-[4α → 8]-3-*O*-galloyl-(+)-epicatechin, 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin, (+)-epicatechin-[4α → 8]-3-*O*-galloyl-(+)-epicatechin, (+)-epicatechin-[4α → 6]-(+)-epicatechin), one known dimer ((+)-epicatechin-[4α → 8]-(+)-epicatechin), three known monomers ((+)-epicatechin, 3-*O*-galloyl-(+)-epicatechin, catechin) and gallic acid were isolated from *Byrsonima crassifolia* bark, which is used medicinally by the Mixe Indians (Oaxaca, Mexico). The new compounds were characterized by spectroscopic methods, as well as by thiolytic degradation with toluene-α-thiol and structural elucidation of the cleavage products by ^{1}H NMR and OR measurements. Bonding positions of dimeric compounds were determined by ^{1}H NMR spectroscopy of their peracetate derivatives. The backbone of the isolated compounds consisted mainly of flavan-3-ol units with the 2*S*-configuration which so far have been rarely found in nature. The enantiomeric purity of (+)-epicatechin, (+)-epicatechin-[4α → 8]-(+)-epicatechin and (+)-epicatechin-[4α → 6]-(+)-epicatechin was checked by HPLC on a chiral cyclodextrin column.

INTRODUCTION

Byrsonima crassifolia, popularly known as 'nanche', is used medicinally by the Mixe Indians of Oaxaca in Mexico to treat gastrointenstinal disorders [1] and skin infections [2]. Similar uses are reported from other parts of Central America [3–5]. The EtOH–H_2O extract of the bark showed good antiinflammatory activity in the HET–CAM assay [6] and the cyclooxygenase-inhibition assay [7]. Furthermore, leaf and bark extracts displayed spasmogenic effects on rat fundus [8] and antimicrobial activity [4, 5]. The only compounds isolated from *B. crassifolia* so far are β-amyrin [9] and 1,2-di-*O*-palmitoyl-3-*O*-(6-sulpho-α-D-quinovopyranosyl)-glycerol [10]. The volatile components of the fruit have been analysed by gas chromatography [11]. Because gastrointestinal disorders in tropical countries may be caused by intestinal parasites we tested extracts of this plant for nematodicidal activity. Guided by this assay we investigated the EtOAc-soluble fraction of an EtOH–H_2O extract of the bark phytochemically. This paper deals with the isolation and structural elucidation of proanthocyanidins consisting mainly of (+)-epicatechin units, which only rarely have been found in plants [12].

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RESULTS AND DISCUSSION

Stem bark of *B. crassifolia* was refluxed with EtOH–H_2O mixtures to give a crude extract which after removal of the EtOH was successively extracted with CH_2Cl_2 and EtOAc. In an *in vitro* assay with the free living plant nematode, *Caenorhabditis elegans*, the crude extract inhibited nematode multiplication with an IC_{50} of 175 ppm. The IC_{50} of the H_2O-phase and EtOAc-phase was 250 ppm and 175 ppm, respectively. At 500 ppm, reproduction was inhibited by 91%, 95% and 98% by the crude extract, the H_2O-phase and the EtOAc-phase, respectively. Colour reactions on TLC (orange–red colouration with vanillin–sulphuric acid reagent and green or blue colouration with ferric chloride reagent) of the EtOAc-soluble part suggested that proanthocyanidins were the major constituents. Separation by

CC on Sephadex LH-20 yielded seven fractions (A1–A5, B1 and B2). Acid hydrolysis of these fractions yielded cyanidin as the only anthocyanidin pigment and gallic acid. 2D TLC of all fractions showed characteristic spots which gave either blue or green colourations when sprayed with $FeCl_3$. A green colouration usually indicates the presence of pyrocatechol partial structures, whereas a blue colouration is characteristic for pyrogallol partial structures. Since cyanidin was formed as the only anthocyanidin by acid hydrolysis, the blue colouration suggested the presence of galloyl moieties in the molecules. Fractions A1–A5, B1 and B2 were further purified by CC on MCI-gel or by silicagel CC followed by HPLC on RP-18. This led to the isolation of compounds **1**, **3**, **5**, **6**, **9**, **10**, **12**–**14** and gallic acid.

The new compound **1** exhibited the $[M + H]^+$ peak in the electrospray-mass spectrum at *m/z* 1171, indicating a triflavanoid structure with two galloyl moieties within the molecule. Fragment ions at *m/z* 291, 731, 441 and 881 produced by cleavage of the interflavanoid bonds due to CAD (collision activated decomposition) of $[M + H]^+$ ions in the MS–MS [13] suggested the presence of two 3-*O*-galloyl-flavanol units (catechin or epicatechin) in the upper and middle positions and an unsubstituted catechin or epicatechin unit in the lower position. The H-3 NMR signals corresponding to the middle and upper flavan units appeared at δ5.50 and 5.56, respectively, confirming the position of the galloyl groups. Controlled acid catalysed degradation of **1** with toluene-α-thiol and subsequent separation of the cleavage products by flash-chromatography on MCI-gel followed by CC on Sephadex LH-20 yielded 3-*O*-galloyl-(+)-epicatechin-4α-benzylthioether (**2**) and 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin (**3**) by cleavage of the upper bond together with 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-3-*O*-galloyl-(+)-epicatechin-4α-benzylthioether (**4**) and (+)-epicatechin (**5**) by cleavage of the lower bond. Compounds **2** and **5** were identified by ^{1}H NMR and OR in comparison with literature values. The enantiomer of dimer **3** is referred to in [14] and [15] but spectral data could not be found. Thiolytic degradation of **3**, which was also isolated as a genuine component, yielded (+)-epicatechin (**5**) and 3-*O*-galloyl-(+)-epicatechin-4-α-benzylthioether (**2**). In order to obtain information on the type of interflavan linkage within the dimer, it was peracetylated and then characterized by ^{1}H NMR with the aid of ^{1}H–^{1}H L.R.-COSY. According to Hemingway *et al.* [16], the chemical shift of the A-ring protons of the upper (u) unit readily distinguishes between [4 → 8]- and [4 → 6]-linked peracetylated proanthocyanidin dimers. In addition, Kolodziej [17] assigns a chemical shift of the lower (l) H-2 signal at δ4.37–5.01

Table 1. ^{1}H NMR data of peracetates of **3**, **9** and **10** in $CDCl_3$ (400 MHz; standard: $CHCl_3$ = 7.24 ppm)

	Peracetate of **3**		Peracetate of **9**		Peracetate of **10**	
H	δ [ppm] (*J* [Hz])	^{1}H–^{1}H COSY l.r. Cross peak	δ [ppm] (*J* [Hz])	^{1}H–^{1}H COSY l.r. Cross peak	δ [ppm] (*J* [Hz])	^{1}H–^{1}H COSY l.r. Cross peak
2 l	4.56 *bs*	3 l, 4 l, 2′ l, 6′ l	4.70 *bs*	3 l, 4 l, 2′ l, 6′ l	4.75 *bs*	3 l, 4 l, 2′ l, 6′ l
3 l	5.11 *m*	2 l, 4 l	5.27 *m*	2 l, 4 l	5.30 *m*	2 l, 4 l
4 l	2.88 *m*	2 l, 3 l	3.03 *m*	2 l, 3 l	3.03 *m*	2 l, 3 l
2 u	5.67 *bs*	3 u, 4 u, 2′ u, 6′ u	5.57 *bs*	3 u, 4 u, 2′ u, 6′ u	5.67 *bs*	3 u, 4 u, 2′ u, 6′ u
3 u	5.42 *dd* (1.5; 2.25)	2 u, 4 u	5.23 *dd* (1.0; 3.0)	2 u, 4 u	5.55 *dd* (1.5; 3.0)	2 u, 4 u
4 u	4.55 *d* (2.25)	2 u, 3 u, 6 u	4.40 *d* (3.0)	2 u, 3 u, 6 u	4.49 *d* (3.0)	2 u, 3 u, 6 u
6 l	6.67 *s*		6.66 *s*		6.68 *s*	
6 u	6.05 *d* (2.25)	4 u, 8 u	6.11 *d* (2.25)	4 u, 8 u	6.20 *d* (2.25)	4 u, 8 u
8 u	6.25 *d* (2.25)	6 u	6.23 *d* (2.25)	6 u	6.28 *d* (2.25)	6 u
2′ l	7.03 *d* (2.25)	2 l, 6′ l	7.15 *d* (2.25)	2 l, 6′ l	7.18 *d* (2.25)	2 l, 6′ l
5′ l	7.02 *d* (8.25)	6′ l	7.04 *d* (8.25)	6′ l	7.06 *d* (8.25)	6′ l
6′ l	6.88 *dd* (2.25; 8.25)	2 l, 2′ l, 5′ l	6.93 *dd* (2.25; 8.25)	2 l, 2′ l, 5′ l	6.96 *dd* (2.25; 8.25)	2 l, 2′ l, 5′ l
2′ u	7.31 *bs*	2 u, 6′ u	7.38 *d* (2.25)	2 u, 6′ u	7.31 *d* (2.25)	2 u, 6′ u
5′ u	7.15 *d* (8.25)	6′ u	7.16 *d* (8.25)	6′ u	7.16 *d* (8.25)	6′ u
6′ u	7.30 *dd* (2.25; 8.25)	2 u, 2′ u, 5′ u	7.18 *dd* (2.25; 8.25)	2 u, 2′ u, 5′ u	7.23 *dd* (2.25; 8.25)	2 u, 2′ u, 5′ u
H-gall	7.54 *s*		7.66 *s*		7.54 *s* 7.69 *s*	

l = lower unit, u = upper unit.

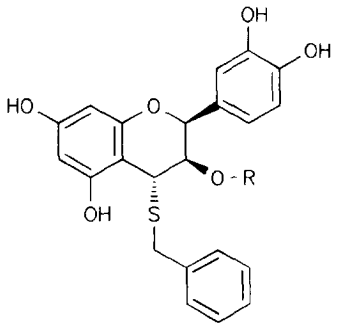
to the [4 → 8]-linked dimer-peracetate compared to a chemical shift at δ5.04–5.35 for the same proton of the [4 → 6] isomer. The chemical shifts of H-6(u), H-8(u) and H-2(1) at δ6.05, 6.25 and 4.56, respectively, are thus typical for a [4 → 8] linkage. ^1H–^1H L.R.-COSY led to the assignment of all ^1H signals in the ^1H NMR spectra of the peracetate derivative except for those due to H-6 and H-8 in the upper unit. The ^1H signals due to H-2, H-3 and H-4 of the C-rings as well as H-2′, H-5′, and H-6′ of the B-rings are evidently correlated with each other and the combination of rings B and C in the epicatechin unit is revealed by the appearance of a significant long-range ^1H–^1H correlation of H-2 with H-2′ and H-6′ within the same flavan unit (Table 1). Furthermore, H-4(u) is correlated with H-6 of the upper unit. These correlations could not be detected in the spectra of the free phenolic proanthocyanidins probably due to broadening and overlapping of signals. Dimer **3** was thus identified as 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin. The bonding position of **4** was established by interpreting the ^1H NMR spectrum of its peracetate derivative with the aid of ^1H–^1H L.R.-COSY (Table 2). The chemical shifts of H-6 and H-8 of the upper flavanoid unit at δ6.26 and 6.31 are typical for a [4 → 8] linkage of the units [16]. The trimer was thus identified as 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin.

The new compound **6** yielded 3-*O*-galloyl-(−)-epicatechin-4β-benzylthioether (**7**), 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin (**3**), 3-*O*-galloyl-(−)-epicatechin-[4β → 8]-3-*O*-galloyl-(+)-epicatechin-4α-benzylthioether (**8**) and (+)-epicatechin (**5**) by partial thiolytic degradation and subsequent separation of the cleavage products by flash-chromatography on MCI-gel and CC on Sephadex LH-20. Compound **7** was identified by ^1H NMR and OR in comparison with literature values and **3** and **5** were identical with the samples obtained from **1**. The bonding position of **8** was established as described for **4**. The chemical shifts of H-6 and H-8 of the upper flavanoid unit at δ6.16 and 6.27 are as those of **4** typical for a [4 → 8] linkage of the units [16]. Compound **6** was thus identified as 3-*O*-galloyl-(−)-epicatechin-[4β → 8]-3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin.

Table 2. ^1H NMR data of peracetates of **8**, and **4** in $CDCl_3$ (400 MHz; standard: $CHCl_3$ = 7.24 ppm)

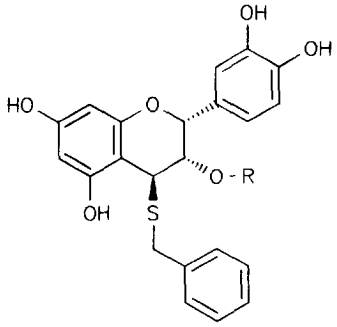
	Peracetate of **8**		Peracetate of **4**	
H	δ [ppm] (*J* [Hz])	^1H–^1H COSY l.r. Cross peak with	δ [ppm] (*J* [Hz])	^1H–^1H COSY l.r. Cross peak with
2 l	5.28 *bs*	3 l, 4 l, 2′ l, 6′ l	5.31 *bs*	3 l, 4 l, 2′ l
3 l	5.22 *m*	2 l, 4 l	5.25 *bs*	2 l, 4 l
4 l	4.37 *d* (1.8)	2 l, 3 l	4.36 *d* (1.5)	2 l, 3 l
2 u	5.50 *bs*	3 u, 4 u, 2′ u, 6′ u	5.59 *bs*	3 u, 4 u, 2′ u
3 u	5.25 *dd* (1.65; 3.0)	2 u, 4 u	5.60 *m*	2 u, 4 u
4 u	4.40 *d* (3.0)	2 u, 3 u	4.47 *d* (3.15)	2 u, 3 u
6 l	6.64 *s*		6.62 *s*	
6 u	6.16 *d* (2.25)	8 u	6.26 *d* (2.25)	8 u
8 u	6.27 *d* (2.25)	6 u	6.31 *d* (2.25)	6 u
2′ l	7.18 *d* (2.25)	2 l, 6′ l	7.20 *d* (2.25)	2 l, 6′ l
5′ l	7.08 *d* (8.25)	6′ l	7.08 *d* (8.25)	6′ l
6′ l	6.95 *dd* (2.25; 8.25)	2′ l, 5′ l	6.98 *dd* (2.25; 8.25)	2′ l, 5′ l
2′ u	7.35 *d* (1.8)	2 u, 6′ u	7.28 *d* (2.25)	2 u, 6′ u
5′ u	7.16 *d* (8.25)		7.16 *d* (8.25)	
6′ u	7.15 *dd* (2.25; 8.25)	2′ u	7.15 *dd* (2.25; 8.25)	2′ u
H-gall	7.70 *s*		7.54 *s*; 7.75 *s*	
S-CH_2	3.93 *d*; 4.15 *d* (13.5 each)		3.93 *d*; 4.16 *d* (13.5 each)	
H-benzylic ring	7.25–7.45		7.25–7.45	

l = lower unit, u = upper unit.



11 R = H

2 R = galloyl



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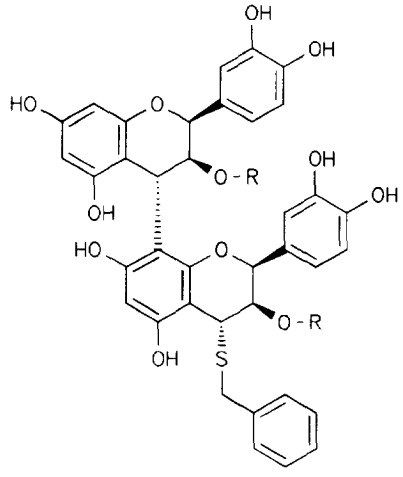
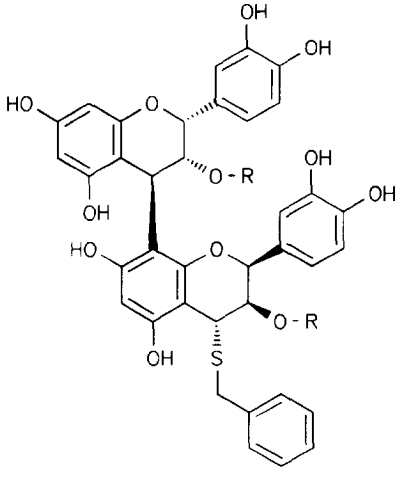


4 R = galloyl

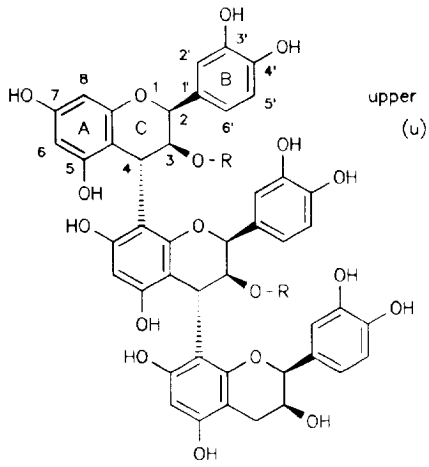


8 R = galloyl

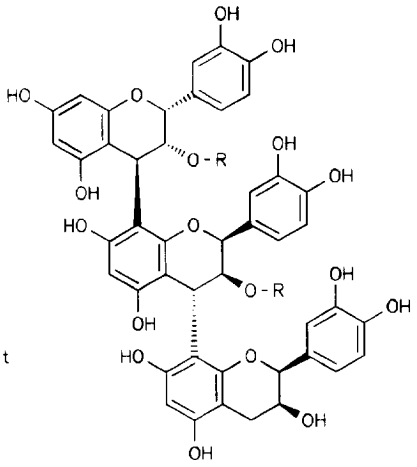
Compounds **9** and **10** were characterized as described for **3** and thus identified as (+)-epicatechin-[4α → 8]-3-*O*-galloyl-(+)-epicatechin (**9**) and 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-3-*O*-galloyl-(+)-epicatechin (**10**). Thiolytic degradation of **9** yielded (+)-epicatechin-4α-benzylthioether (**11**) and 3-*O*-galloyl-(+)-epicatechin (**12**) and thiolysis of **10** gave 3-*O*-galloyl-(+)-epicatechin-4α-benzylthioether (**2**) and **12**, which were identified by ^{1}H NMR and OR in comparison with literature data.

(+)-Epicatechin-[4α → 8]-(+)-epicatechin (**13**), (+)-epicatechin-[4α → 6]-(+)-epicatechin (**14**), (+)-epicatechin (**5**) and 3-*O*-galloyl-(+)-epicatechin (**12**) were identified by ^{1}H NMR and OR in comparison with literature values. Identification of catechin and gallic acid was carried out by HPLC addition analysis with authentic samples.

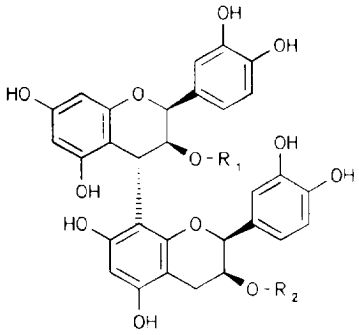
OR measurements of some of the proanthocyanidin samples exhibited significantly smaller values than those reported for pure enantiomers. Since the differences might be due to the presence of different amounts of both enantiomers we tried to check the enantiomeric purity by chiral HPLC. We were encouraged by the report of Kashiwada *et al.* [12], who had successfully separated enantiomeric mixtures of epicatechin-[4 → 8]-epicatechin and epicatechin by HPLC on chiral cellulose columns. For **5**, **13** and **14** authentic material with known enantiomeric configuration was available (Michaela Hör, Institut für Pharmazeutische Biologie, Freiburg: preliminary results of doctoral thesis). With these samples we established HPLC conditions for the separation on a chiral cyclodextrin column. Compounds **5** and **14** proved to be pure 2*S*-enantiomers, whereas **13** was a mixture of (+)-epicatechin-[4α → 8]-(+)-epicatechin with smaller amounts of (−)-epicatechin-[4β → 8]-(−)-epicatechin. The galloylated compounds showed strong tailing and since authentic material with the enantiomeric configuration was not available, optimal separation con-



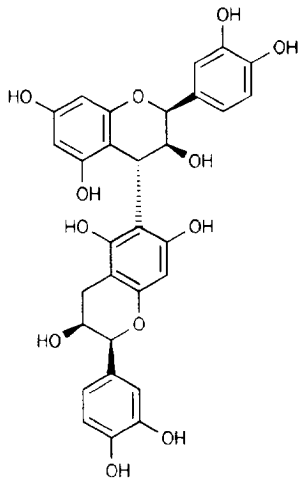
1 R = galloyl



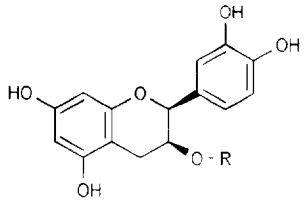
6 R = galloyl



13 $R_1 = R_2 = H$
3 R_1 = galloyl, $R_2 = H$
9 $R_1 = H$, R_2 = galloyl
10 $R_1 = R_2$ = galloyl



14



5 R = H
12 R = galloyl

ditions could not be established. Configurations of these compounds were therefore deduced from OR measurements only.

EXPERIMENTAL

Plant material. Stem bark of a *ca.* 20-year-old tree was harvested in November 1992 in Oaxaca, Mexico and identified as *Byrsonima crassifolia* Kunth (Malpighiaceae) by M. Heinrich. Voucher specimens (Heinrich and Antonio: GUI 65) of the plant are deposited at the herbarium of the Institut für Pharmàzeutische Biologie, Freiburg.

General. ^{1}H NMR were recorded at 400 MHz, chemical shifts are given in δ (ppm). 2D NMR spectra (^{1}H–^{1}H COSY and long-range ^{1}H–^{1}H COSY) were measured by the use of a standard COSY pulse sequence

and L.R. COSY was optimized for coupling of 2 Hz. FAB-MS were obtained in the positive mode; matrix: HOAc–1,4-butanediol–glycerol (2:2:1); primary ions: Cs, acceleration with 9 kV; secondary ions were accelerated with 6 kV. ESI-MS: instrument equipped with an electrospray source; 4.2 keV for full scan; N_2 sheath gas; CAD: Ar 1 mtorr; 1 μg sample μl^{-1} in MeOH–H_2O (1:1); 5 μl direct-loop injections with a flow of 5$\mu l\,min^{-1}$ of MeOH–H_2O (1:1). Optical rotations (OR) were recorded at 26 or 28° using Me_2CO solns in cells of path length 1 dm and volume 1 ml. HPLC was carried out on an Eurosphere C-18 column (7 μm; 250 × 8 mm) and on Li Chro Cart 250-4 Chira Dex (5 μm; 100Å; 300–360 $m^2\,g^{-1}$); detection: UV 280 nm. For CC, Sephadex LH-20, 25–100 μm (Pharmacia) and MCI-gel CHP-20P, 75–150 μm (Mitsubishi Chem. Ind.) were used. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck) with EtOAc–HCO_2H–H_2O, 18:1:1 (system C) and spots were detected by spraying with vanillin–sulphuric acid and $FeCl_3$ reagents. Cellulose o.F. (Merck) with HOAc–conc. HCl–H_2O (30:3:10) (Forestal) as eluent was used after acid hydrolysis and 2D TLC was carried out on Cellulose F (Merck) with HOAc 6% (system A) and 2-BuOH–HOAc–H_2O, 14:1:5 (system B) and spraying with $FeCl_3$ reagent (1%).

Acid hydrolysis. Samples of 1 mg of each proanthocyanidin fr. were dissolved in 0.2 ml *n*-BuOH–conc. HCl (19:1) and 5μl of a 2% (w/v) soln of a ferric reagent [$(NH_4)FeIII(SO_4)_2 \times 24\,H_2O$] in 2N HCl. The mixts were sealed in 1 ml glass vials and kept for 60 min at 100°. The solns were then examined by TLC on cellulose with Forestal solvent and pigment zones were compared with those of commercial anthocyanidins and gallic acid.

Acetylation. Solns of compounds in pyridine–Ac_2O (25 mg sample: 1 ml pyridine: 1 ml Ac_2O) were kept at room temp. with stirring for 48 hr. Excess reagent was decomposed by addition of ice H_2O and the resulting ppt collected by filtration.

Extraction and isolation. Air-dried and powdered stem bark (500 g) was refluxed with 96% EtOH (2.5 l) and 70% EtOH (2 × 2, 5 l) and the EtOH removed *in vacuo*. The aq. residue was freeze-dried to yield 170 g of crude extract. The latter was dissolved in 1.7 l H_2O, washed with CH_2Cl_2 (3 × 1.7 l) and extracted with EtOAc (4 × 1.7 l). The EtOAc-phase after removal of solvent and the H_2O-phase were lyophilysed to yield 29 g and 93.5 g, respectively. The EtOAc extract (16 g) was chromatographed with EtOH (column A: 560 × 50 mm) and EtOH–H_2O–Me_2CO (8.5:8.5:3; column B: 600 × 50 mm) on Sephadex LH-20. Frs were monitored by TLC (C) and grouped as follows: 1556–1828 ml = A1; 1829–2500 ml = A2; 3005–3949 ml = A3; 4475–5944 ml = A4; 6705–7834 ml = A5. The remaining compounds were washed off the Sephadex column with Me_2CO–H_2O (4:1), the solvent evapd and the residue rechromatographed on Sephadex LH-20 with EtOH–H_2O–Me_2CO, yielding B1 = 4356–5045 ml and B2 = 5046–5645 ml. Fr. A1(96 mg) was then chromatographed on silica gel 60 (200 × 10 mm; 5 ml frs) with CH_2Cl_2–MeOH–H_2O (70:30:3). Frs 7–20 were combined and after removal of solvent purified by HPLC (column: Eurosphere RP-18; 7μm; 250 × 8 mm; solvent A: 2.2% MeOH, pH 2.6 (CF_3CO_2H); solvent B: 100% MeOH; 30% B isocratic for 15 min; 30% B to 100% B linear for 15 min; flow rate 2 $ml\,min^{-1}$; detection: 280 nm; R_t 6.51 min) to yield gallic acid (9 mg). Fr. A2 (1022 mg) was further purified on MCI-gel with MeOH 50% (column: 370 × 17,5 mm; 20 ml frs) and yielded (+)-epicatechin (**5**) (700 mg) in frs 7–11. Fr. 6 (150 mg) contained a mixt. of epicatechin and catechin. Fr. A3 (1012 mg) was also purified on MCI-gel with MeOH 35% (column: 410 × 17.5 mm; 18 ml fractions) to give (+)-epicatechin-[4α → 8]-(+)-epicatechin (**13**) (500 mg) (frs 13–30) and 3-*O*-galloyl-(+)-epicatechin (**12**) (190 mg) (frs 58–88). CC of fr. A4 (1075 mg) on MCI-gel with MeOH 35% (column: 430 × 17.5 mm; 20 ml frs) yielded 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin (**3**) (450 mg) in frs 10–16 and subsequent elution with MeOH gave a fr. which was further purified by CC on MCI-gel with 50% MeOH (column: 370 × 17.5 mm; 20 ml frs) to yield (+)-epicatechin-[4α → 6-(+)-epicatechin (**14**) (50 mg) in frs 11–17. Purification of fr. A5 (1088 mg) on MCI-gel with MeOH 35% (column: 410 × 17.5 mm; 21 ml frs) yielded 3-*O*-galloyl-(−)-epicatechin-[4β → 8]-3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin (**6**) (85 mg) in frs 8 and 9. Rechromatography of frs 17–30 on MCI-gel with 40% MeOH (column: 370 × 17.5 mm; 21 ml frs) yielded (+)-epicatechin-[4α → 8]-3-*O*-galloyl-(+)-epicatechin (**9**) (167 mg) in frs 12–20. Fr. B1(765 mg) was further purified on MCI-gel with MeOH 35% (column: 370 × 17.5 mm; 15 ml frs). Frs 8–11 were combined to yield 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin (**1**) (220 mg). CC of fr. B2 (1113 mg) on MCI-gel with MeOH 35% (column: 490 × 17.5 mm; 20 ml frs) yielded 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-3-*O*-galloyl-(+)-epicatechin (**10**) (136 mg) in frs 32–50.

General procedure for thiolytic degradation and work-up of cleavage products

Dimers. For thiolytic degradation of dimers' samples were dissolved in EtOH. Toluene-α-thiol and HOAc were added under N_2 and the vial sealed and kept at 90° for 24 hr (10 mg sample: 1 ml EtOH: 50 μl toluene-α-thiol: 20 μl HOAc). After evapn of solvent, the oily residue was flash-chromatographed on RP-18-Varian Mega Bond Elut (1 g/6 ml column) with MeOH (35% → 100%; 5% steps). Thus, the thioethers (**11** and **2**) were isolated and identified by ^{1}H NMR and OR measurements in comparison with lit. values and authentic samples. The flavan-3-ols eluted together with the uncleaved dimers and were subsequently sepd by flash-CC on Si Viran Mega Bond Elut (1 g/6 ml column) with CH_2Cl_2–MeOH–H_2O (70:30:3) (0.8 ml frs). Identification was carried out by ^{1}H NMR and OR in comparison with authentic samples.

Trimers. As described for dimers but reaction time was only 14 hr at 90°. After evapn of solvent, the oily residue

was flash-chromatographed on MCI-gel (30 × 10 mm) with MeOH (15% → 100%; 5% steps) to yield 2 main frs, I (Ia) and II (IIa). Fr. I (Ia) contained the uncleaved trimer (**1** in I; **6** in Ia), a dimer (**3** in I and Ia) and a monomer (**5** in I and Ia). Fr. II (IIa) contained the monomeric (**2** in II and **7** in IIa) and dimeric thioethers (**4** in II and **8** in IIa). Each fr. was separately chromatographed on Sephadex LH-20 with EtOH as eluent. The cleavage products were characterized by ^{1}H NMR of the free phenols and their peracetate derivatives as well as by OR.

Sepn of enantiomers. Compounds **5**, **13** and **14** (isolated) and their authentic enantiomers were sepd on Chira Dex with MeOH–H_2O mixts at pH 3.8 (addition of 0.1% NEt_3; pH adjusted with HOAc); flow rate: 0.8 ml min^{-1}. Conditions were as follows. Epicatechin: MeOH 20% (pH 3.8) R_t (+)-epicatechin (**5**): 19.8 min; R_t (−)-epicatechin: 18.81 min. Epicatechin-[4 → 8]-epicatechin: MeOH 10% (pH 3.8) R_t (+)-epicatechin-[4α → 8]-(+)-epicatechin (**13**): 8.05 min; R_t (−)-epicatechin-[4β → 8]-(−)-epicatechin: 9.02 min. Epicatechin-[4 → 6]-epicatechin: MeOH 20% (pH 3.8) R_t (+)-epicatechin-[4α → 6]-(+)-epicatechin (**14**): 25.58 min; R_t (−)-epicatechin-[4β → 6]-(−)-epicatechin: 26.79 min.

3-O-*Galloyl*-(+)-*epicatechin*-[4α → 8]-3-O-*galloyl*-(+)-*epicatechin*-[4α → 8]-(+)-*epicatechin* (**1**). $[\alpha]_D^{26}$ − 42° (Me_2CO; *c* 1.0). R_f (A) 0.50, (B) 0.33, (C) 0.19. ESI-MS: *m/z* 1171 $[M + H]^+$; MS–MS of $[M + H]^+$: *m/z* 1171 $[M + H]^+$, 1019, 881, 731, 711, 591, 541, 441, 291, 271, 153, 139. ^{1}H NMR (acetone-d_6/D_2O, standard: acetone-d_5 = 2.04 ppm): δ2.59 (1H, *m*, H-4β(1)), 2.87 (1H, *dd*, *J* = 4.65 Hz, 16.5 Hz, H-4α(1)), 4.30 (1H, *bs*, H-3(1)), 4.74 (1H, *bs*, H-4(m or u)), 4.82 (1H, *bs*, H-4(m or u)), 5.09 (1H, *bs*, H-2(1)), 5.50 (2H, *bs*, H-2(m or u) and H-3(m or u)), 5.56 (1H, *dd*, *J* = 2.25 Hz, 3.75 Hz, H-3(m or u)), 5.62 (1H, *bs*, H-2 (m or u)), 5.88–6.15 (4H, A-ring-H), 6.45 (1H, *d*, *J* = 8.25 Hz, H-5'), 6.60 (1H, *d*, *J* = 8.25 Hz, H-5'), 6.67 (1H, *d*, *J* = 8.25 Hz, H-5'), 6.72 (1H, *dd*, *J* = 2.25 Hz, 8.25 Hz, H-6'), 6.77–7.05 (9H, remaining B-ring- and galloyl-H). Partial thiolytic degradation yielded 3-*O*-galloyl-(+)-epicatechin-4α-benzylthioether (**2**), 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin (**3**), 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-3-*O*-galloyl-(+)-epicatechin-4α-benzylthioether (**4**) and (+)-epicatechin (**5**).

3-O-*Galloyl*-(+)-*epicatechin*-4α-*benzylthioether* (**2**). $[\alpha]_D^{28}$ + 84° (Me_2CO; *c* 0.725) (from **1**); $[\alpha]_D^{28}$ + 83° (Me_2CO; *c* 0.445) (from **3**); $[\alpha]_D^{28}$ + 41° (Me_2CO; *c* 0.245) (from **10**); lit. [18] for 3-*O*-galloyl-(−)-epicatechin-4β-benzylthioether: $[\alpha]_D^{20}$ − 48° (Me_2CO; *c* 0.5). ^{1}H NMR data were consistent with reported values for the (−)-enantiomer [19]. The differences of optical rotations may be due to enantiomeric or unspecific impurities. Nevertheless, the strong positive rotation of all samples shows that the 2*S*-enantiomer was at least predominating.

3-O-*Galloyl*-(+)-*epicatechin*-[4α → 8]-(+)-*epicatechin* (**3**). $[\alpha]_D^{26}$ + 18° (Me_2CO; *c* 0.96). R_f (A) 0.60, (B) 0.44, (C) 0.44. FAB-MS: *m/z* 731 $[M + H]^+$. ^{1}H NMR (acetone-d_6, standard: acetone-d_5 = 2.04 ppm): δ2.68 (1H, *dd*, *J* = 4.5 Hz, 16.5 Hz, H-4β(1)), 2.88 (1H, *dd*, *J* = 4.65 Hz, 16.5 Hz, H-4α(1)), 4.16 (1H, *bs*, H-3(1)), 4.67 (2H, *bs*, H-2(1) and H-4(u)), 5.35 (1H, *bs*, H-2(u)), 5.59 (1H, *bs*, H-3(u)), 5.91 and 6.09 (3H, H-6(u), H-6(1), H-8(u)), 6.63 (1H, *d*, *J* = 8.25 Hz, H-5'), 6.67 (1H, *d*, *J* = 8.25 Hz, H-5'), 6.75 (1H, *dd*, *J* = 2 Hz, 8.25 Hz, H-6'), 6.84–7.0 (5H, remaining B-ring- and galloyl-H). Partial thiolytic degradation yielded 3-*O*-galloyl-(+)-epicatechin-4α-benzylthioether (**2**) and (+)-epicatechin (**5**). Since the ^{1}H NMR spectra of **3** from **1** and **6** were identical with that of **3** isolated from fr. A4, the middle unit of **1** and **6** is concluded to possess the 2*S*-configuration.

3-O-*Galloyl*-(+)-*epicatechin*-[4α → 8]-3-O-*galloyl*-(+)-*epicatechin*-4α-*benzylthioether* (**4**). ^{1}H NMR of its peracetate derivative in Table 2. The absolute configuration of both units is concluded to be 2*S*, since the 2*S*-configuration of the upper and middle unit of **1**, which **4** is derived from, was independently proven by thiolytic degradation of **1** to **2** and **3**.

(+)-*Epicatechin* (**5**). $[\alpha]_D^{28}$ + 45° (Me_2CO; *c* 0.888) (from **1**); $[\alpha]_D^{28}$ + 36° (Me_2CO; *c* 0.195) (from **3**); $[\alpha]_D^{28}$ + 33° (Me_2CO; *c* 0.905) (from **6**); $[\alpha]_D^{26}$ + 49° (Me_2CO; *c* 1.195) (from fr. A2); lit. [20] for (−)-epicatechin: $[\alpha]_D^{22}$ − 52° (Me_2CO; *c* 0.54), commercially available (−)-epicatechin (Roth):. $[\alpha]_D^{28}$ − 49° (Me_2CO; *c* 0.99). R_f (A) 0.40, (B) 0.51, (C) 0.63. ^{1}H NMR data were consistent with published values [21]. HPLC addition analysis of our samples obtained from **3** and **6** with (+)- and (−)-epicatechin on Chira Dex showed that only the (+)-enantiomer was detectable. Thus, the low value for OR compared to the commercially available standard is probably due to unspecific impurities.

3-O-*Galloyl*-(−)-*epicatechin*-[4β → 8]-3-*O*-*galloyl*-(+)-*epicatechin*-[4α → 8]-(+)-*epicatechin* (**6**). $[\alpha]_D^{26}$ − 56° (Me_2CO; *c* 1.095). R_f (A) 0.50, (B) 0.23, (C) 0.29. The ^{1}H NMR spectrum was very complex due to broadening and overlapping of signals. Partial thiolytic degradation yielded 3-*O*-galloyl-(−)-epicatechin-4β-benzylthioether (**7**), 3-*O*-galloyl-(−)-epicatechin-[4β → 8]-3-*O*-galloyl-(+)-epicatechin-4α-benzylthioether (**8**), 3-*O*-galloyl-(+)-epicatechin-[4α → 8]-(+)-epicatechin (**3**) and (+)-epicatechin (**5**).

3-O-*galloyl*-(−)-*epicatechin*-4β-*benzylthioether* (**7**). $[\alpha]_D^{28}$ − 46° (Me_2CO; *c* 0.87), lit. [18]: $[\alpha]_D^{20}$ − 48° (Me_2CO; *c* 0.5). ^{1}H NMR was consistent with reported values [19].

3-O-*Galloyl*-(−)-*epicatechin*-[4β → 8]-3-*O*-*galloyl*-(+)-*epicatechin*-4α-*benzylthioether* (**8**). ^{1}H NMR of its peracetate derivative in Table 2. The absolute configurations of the units are derived from the absolute configurations of **7** and **3**, the other cleavage products of **6**.

(+)-*Epicatechin*-[4α → 8]-3-O-*galloyl*-(+)-*epicatechin* (**9**). $[\alpha]_D^{26}$ + 12.5° (Me_2CO; *c* 1.042), lit. [22] for (−)-enantiomer: $[\alpha]_D^{19}$ − 45.8° (Me_2CO; *c* 0.72). R_f (A) 0.51, (B) 0.40, (C) 0.50. FAB-MS: *m/z* 731 $[M + H]^+$. ^{1}H NMR data were consistent with published values [23]. For ^{1}H NMR of the peracetate derivative see Table 1. Partial thiolytic degradation yielded (+)-epicatechin-4α-benzylthioether (**11**) and 3-*O*-galloyl-(+)-epicatechin (**12**).

3-O-*Galloyl*-(+)-*epicatechin*-[4α → 8]-3-O-*galloyl*-(+)-*epicatechin* (**10**). $[\alpha]_D^{26}$ + 13.4° (Me_2CO; *c* 1.19), lit. [19] for (−)-enantiomer: $[\alpha]_D^{31}$ − 93.8° (Me_2CO; *c* 1.0). R_f (A) 0.43, (B) 0.46, (C) 0.40. FAB-MS: *m/z* 883 $[M + H]^+$. ^1H NMR data consistent with published values [19]. For ^1H NMR of the peracetate derivative see Table 1. Partial thiolytic degradation yielded 3-*O*-galloyl-(+)-epicatechin-4α-benzylthioether (**2**) and 3-*O*-galloyl-(+)-epicatechin (**12**). The great difference in OR of **10** and lit. values are probably due to unspecific impurities since the OR values of the cleavage products are in good agreement with the lit.

(+)-*Epicatechin-4α-benzylthioether* (**11**). $[\alpha]_D^{28}$ + 29° (Me_2CO; *c* 0.31), lit. [19] for (−)-epicatechin-4β-benzylthioether: $[\alpha]_D^{19}$ − 28° Me_2CO; *c* 1.0). ^1H NMR data were in agreement with lit. values [24].

3-O-*galloyl*-(+)-*epicatechin* (**12**). $[\alpha]_D^{28}$ + 75° (Me_2CO; *c* 0.16), (from **9**); $\alpha]_D^{28}$ + 160° (Me_2CO; *c* 0.063) (from **10**); $[\alpha]_D^{26}$ + 161° (Me_2CO; *c* 0.954) (from fr. A3); lit. [25] for 3-*O*-galloyl-(−)-epicatechin: $[\alpha]_D^{20}$ − 160.6° (Me_2CO; *c* 0.22). R_f (A) 0.38, (B) 0.66, (C) 0.63. ^1H NMR data consistent with published values [25]. The rather low value for the OR of the sample from **9** may be due to smaller amounts of the enantiomer or to unspecific impurities.

(+)-*Epicatechin*-[4α → 8]-(+)-*epicatechin* (**13**). $[\alpha]_D^{26}$ − 15° (Me_2CO; *c* 1.318), lit. [20] for (−)-epicatechin-[4β → 8]-(−)-epicatechin: $[\alpha]_D^{25}$ + 35.5° (Me_2CO; *c* 1.0). R_f (A) 0.61, (B) 0.43, (C) 0.50. FAB-MS: *m/z* 579 $[M + H]^+$. ^1H NMR data consistent with published values [23]. HPLC on Chira Dex with MeOH 10% (pH 3.8) showed that, in addition to the predominating (+)-epicatechin-[4α → 8]-(+)-epicatechin (R_t = 8.05 min), smaller amounts of (−)-epicatechin-[4β → 8]-(−)-epicatechin (R_t = 9.02 min) were present in the sample. This explains the difference between the value for OR of **13** and that of the lit.

(+)-*Epicatechin*-[4α → 6]-(+)-*epicatechin* (**14**). $[\alpha]_D^{26}$ − 105° (Me_2CO; *c* 0.993), lit. [26] for (−)-epicatechin-[4β → 6]-(−)-epicatechin: $[\alpha]_D^{24}$ + 94.7° (Me_2CO; *c* 1.7). R_f (A) 0.35, (B) 0.38, (C) 0.57. ^1H NMR data consistent with published values [26]. HPLC on Chira Dex with MeOH 20% (pH 3.8) sepd **14** (R_t = 25.58 min) from authentic (−)-epicatechin-[4β → 6]-(−)-epicatechin (R_t = 26.79 min), thus confirming our compound to be the 2*S*,2*S*-enantiomer.

Catechin. Identified by 2D-coTLC (R_f (A) 0.44, R_f (B) 0.57, R_f (C) 0.63) and HPLC addition analysis with authentic substance. HPLC conditions were as follows: Eurosphere RP-18; 7 μm; 250 × 8 mm; mobile phase: MeOH 30%; pH 2.6; flow rate 2 ml min^{-1}; detection 280 nm; R_t 8.91 min. The absolute configuration was not established.

Gallic acid. Identified by 2D-coTLC (R_f (A) 0.45, R_f (B) 0.50, R_f (C) 0.65), HPLC addition analysis with authentic substance (conditions see catechin:R_t 6.43 min) and ^1H NMR (acetone-d_6, standard: acetone-d_5 = 2.4 ppm): δ7.14 ppm (*s*, H-2 and H-6).

Antihelminthic activity. Caenorhabditis elegans was cultured on a solid medium inoculated with *E. coli* OP 50 [27]. The crude extract, H_2O-phase and EtOAc-phase were tested at 125, 250 and 500 ppm in a microwell plate assay [28]. Five days after incubation, the reproduction of the nematodes was observed. The results are based on 2 different expts.

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