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PROANTHOCYANIDIN POLYMERS WITH ANTISECRETORY ACTIVITY AND PROANTHOCYANIDIN OLIGOMERS FROM *GUAZUMA ULMIFOLIA* BARK

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Key Word Index—*Guazuma ulmifolia*; Sterculiaceae; bark; proanthocyanidins; tannins; polymers; gel permeation chromatography; NMR; thiolytic degradation; (−)-epicatechin; peracetates; antisecretory activity.

Abstract—Bioassay-guided fractionation of a crude extract of *Guazuma ulmifolia* bark led to the isolation of polymeric proanthocyanidins which inactivated cholera toxin (CT). The average degree of polymerization (DP) of the active compounds ranged from 14.4 to 32.0. The polymers consisted mainly of (−)-epicatechin units. In polymers of a representative fraction, the flavanol units were connected by [4 → 8] bonds and, less frequently, by [4 → 6] bonds. Inhibition of CT by tannins increased with M_r and conformation flexibility of the tannin molecule. Several known procyanidin oligomers were also isolated. ^{1}H NMR shift rules to distinguish between [4 → 8] and [4 → 6] linked proanthocyanidin peracetates, that have been proposed for dimers, were extended to trimers and a tetramer. A further diagnostic shift parameter to determine the interflavanoid bonding position is presented and the conformation of oligomeric proanthocyanidin peracetates is discussed.

INTRODUCTION

Guazuma ulmifolia is used by the Mixe Indians of Oaxaca (Mexico) to treat diarrhoea [1]. Similar uses are known from other areas of Mexico [2]. The ethanol-extract of the bark (C) inhibits cholera toxin-induced secretion in rabbit distal colon mounted in an Ussing chamber. The antisecretory activity is due to the water-soluble part (W) of C. SDS–PAGE analysis shows that the activity is due to a specific interaction of C with the A-subunit of the toxin. The results of SDS–PAGE and Ussing chamber experiments correspond well. Thus, SDS–PAGE appears to be a reliable method for the bioassay-guided fractionation of C and for the investigation of structure–activity relationships of tannins. Preliminary examination indicated that the active compounds were polymeric proanthocyanidins which exclusively contain epicatechin and catechin units [3]. The present paper deals with the purification, characterization and structure–activity relationships of these polymeric proanthocyanidins. In addition, several known oligomeric proanthocyanidins were isolated from the ethyl acetate layer of C.

RESULTS AND DISCUSSION

Bioassay-guided fractionation of W by column chromatography on Sephadex LH-20 with ethanol–water and ethanol–water–acetone mixtures yielded several fractions containing oligomeric and polymeric proanthocyanidins (W1.1–W3.7). Only the fractions which eluted with ethanol–water–acetone (7:7:6) (W3.1–W3.7) showed high activity against CT in SDS–PAGE.

The weight average molecular weight ($\overline{M}_w$) and the number average molecular weight ($\overline{M}_N$) of the active fractions (W3.1–W3.7) and of some oligomeric fractions (W1.11–W2.7) were determined by gel permeation chromatography (GPC) of the peracetates. The degree of polymerization (DP) was calculated using an average M_r of 500 for one acetylated flavanol unit. To confirm these results $\overline{M}_N$ was determined by complete thiolytic degradation. The cleavage products were quantified by direct HPLC analysis of the reaction mixture (Table 1). For most of the fractions, GPC indicated lower values for DP than complete thiolysis. These differences can in part be attributed to the use of the unpolar chloroform as eluting solvent [4]. A further reason for the differences between the two methods is the use of linear and rigid polystyrene standards for calibration of GPC in the higher M_r region [5]. The GPC values of fractions W2.2, W3.1, W3.2 and W3.7 were higher than those from thiolysis. The GPC value of W3.1 was almost twice as high as the result obtained by thiolysis. This difference might be attributed to the presence of other linkages besides acid labile [4 → 8] and [4 → 6] interflavanoid bonds. Proanthocyanidins with such unusual linkages are known. For example, Nonaka *et al.* [6] isolated dimeric flavan-3-ols

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Table 1. $\overline{M_w}$, $\overline{M_N}$ and DP of proanthocyanidins of *Guazuma ulmifolia* bark

Substance fraction	GPC of peracetates				Complete thiolysis	
	$\overline{M_w}$*	$\overline{M_N}$†	DP‡	PD§	$\overline{M_N}$†	DP‡
Epicatechin	710	640	1.3	1.11		
Procyanidin B2	1010	939	1.9	1.08	607	2.1±0
Procyanidin C1	1571	1444	2.9	1.09	866	3.0±0.1
W 1.11	3787	2719	5.4	1.39	1788	6.2±0.2
W 1.12	4121	3244	6.5	1.27	1903	6.6±0.5
W 1.13	3620	2906	5.8	1.25	2018	7.0±0.2
W 2.2	7912	5580	11.2	1.42	2623	9.1±0.2
W 2.3	5518	4462	8.9	1.24	2450	8.5±0.3
W 2.4	4825	3934	7.9	1.23	2479	8.6±0.4
W 2.5	4868	4021	8.0	1.21	2680	9.3±0.7
W 2.6	5700	4749	9.5	1.20	3026	10.5±0.1
W 2.7	7159	5667	11.3	1.26	3458	12.0±0.6
W 3.1	22039	15986	32.0	1.38	5100	17.7±0.7
W 3.2	10775	8554	17.1	1.26	4466	15.5±0.1
W 3.3	9125	7216	14.4	1.26	4869	16.9±0.1
W 3.4	10102	7738	15.5	1.31	5042	17.5±1.6
W 3.5	10066	7359	14.7	1.37	5157	17.9±1.4
W 3.6	13932	10525	21.1	1.32	5791	20.1±1.3
W 3.7	15025	10535	21.1	1.43	5071	17.6±0.2

*$\overline{M_w}$: weight average molecular weight.
†$\overline{M_N}$: number average molecular weight.
‡DP: average degree of polymerization.
§PD: polydispersitivity ($\overline{M_w}/\overline{M_N}$).

linked at the B-rings from green tea leaves. The content of such compounds in tea is drastically increased by polyphenol oxidases during standing in air after harvest [7]. Bonds between two benzene rings might also be generated during extraction of the plant material. Tanaka *et al.* [8] showed that the loss of astringency of persimmon fruits during the anaerobic treatment of the flesh with 30% ethanol is due to condensation of the B-rings of proanthocyanidin oligomers with acetaldehyde to form insoluble polymers. To investigate whether the compounds of W3.1 were generated during isolation we repeated the extraction and separation of the polymeric proanthocyanidins under mild conditions; there were no significant qualitative or quantitative differences compared with the first isolation. Three polymeric fractions of the acetone percolate were analysed by GPC and complete thiolysis. These results and the results of the corresponding fractions of the ethanol extract are given in Table 2. The average DP of AW 3.1, as determined by GPC, was also twice as high as the DP obtained by complete thiolysis. Therefore, formation of these compounds with unusual linkages during extraction can be excluded. They might be either genuine compounds or have been generated during drying of the bark [7].

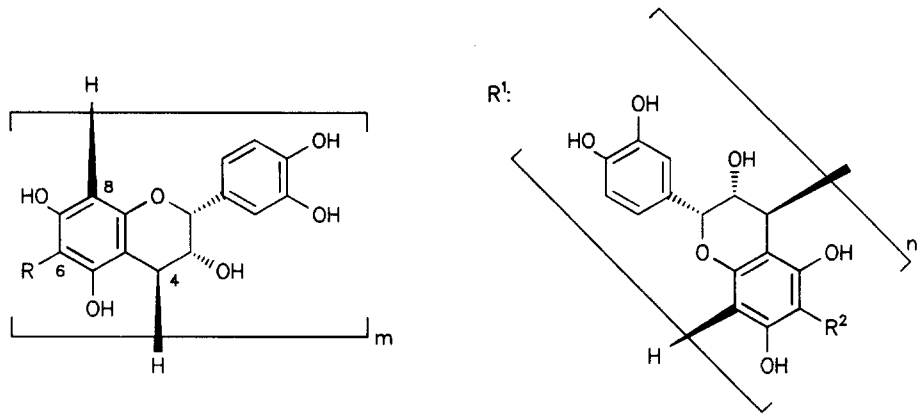
The nature of the extension units of the proanthocyanidin polymers was deduced by complete thiolysis and HPLC analysis of the cleavage products. The major chain unit is (−)-epicatechin (**1**). (+)-Catechin (**11**) comprises 10% as terminal units and 8% as extension units. The type of interflavanoid bonds of the polymers of fraction W 3.3 was determined by partial thiolysis and HPLC identification and quantification of the dimeric thioethers (−)-epicatechin-[4$\beta \rightarrow$8]-(−)-epicatechin-4β-benzylthioether (**5**) and (−)-epicatechin-[4$\beta \rightarrow$6]-(−)-epicatechin-4β-benzylthioether (**6**). The dimers **5** and **6** were found in a ratio of 3:1. Thus, the flavanol units were connected by [4$\rightarrow$8] bonds and, less frequently, by [4$\rightarrow$6] bonds. According to Porter *et al.* [9], the [4$\rightarrow$6] linkage is cleaved at a slower rate than the [4$\rightarrow$8] bond. Therefore, the frequency of [4$\rightarrow$6] linkages in the polymer would be somewhat overestimated if determined by thiolysis, and may be less than 25%. The structure of the polymers of fraction W 3.3 is illustrated in Fig. 1.

To investigate structure–activity relationships of tannins we compared the activity of procyanidins with different DP and the commercially available gallotan-

Table 2. DP of some polymer fractions of *Guazuma ulmifolia* bark obtained by percolation with acetone–water (7:3) compared with the data for the corresponding fractions of the ethanol extract

Fraction	Complete thiolysis	GPC of peracetates	
		DP	PD
AW 3.1	19.7±1.1	40	1.47
W 3.1	17.7±0.7	32	1.38
AW 3.4	15.7±0.6	15	1.34
W 3.4	17.5±1.6	15.5	1.31
AW 3.6	19.8±1.2	20	1.47
W 3.7	17.6±0.2	21.1	1.43

DP: average degree of polymerization.
PD: polydispersitivity ($\overline{M_w}/\overline{M_N}$).



R = H or R = R¹

R² = H or R² = R¹

m = 1 – 12; n = 0 – 12

m + n = 15

1 – 2 units/molecule are (+)–catechin (11)

Configuration at C–4 of catechin extension units presumably predominantly 3,4–*trans* [29].

Fig. 1. Structure of proanthocyanidins of fraction W3.

nin, tannic acid, using SDS–PAGE. Procyanidins with an average DP of 5 are inactive up to 2500 μg. Procyanidins with an average DP of 10 completely bound the A-subunit of the toxin in a dose of 500–1000 μg. Polymers with an average DP of 15 showed high activity with an active dose of 30 μg. Tannic acid inactivated the A-subunit at 500–1000 μg. Thus, the toxin-binding activity of condensed tannins increased with their M_r. Activity may also be dependent on the conformation flexibility of the tannin molecule as the more flexible tannic acid with an average M_r of 940–1852 is as active as the procyanidin decamer with a M_r of 2900. These findings are in good agreement with earlier general observations on the affinity of tannins for proteins [10].

From the ethyl acetate layer the monomer (−)-epicatechin (**1**), the dimers procyanidin B2 (**3**) and procyanidin B5 (**4**), the trimers procyanidin C1 (**7**), (−)-epicatechin-[4β→6]-(−)-epicatechin-[4β→8]-(−)-epicatechin (**8**) and (−)-epicatechin-[4β→8]-(−)-epicatechin-[4β→6]-(−)-epicatechin (**9**) and the tetramer, (−)-epicatechin-[4β→8]-(−)-epicatechin-[4β→8]-(−)-epicatechin-[4β→8]-(−)-epicatechin (**10**) were isolated. All compounds are known from nature. Compounds **1** and **3** were identified by ^{1}H NMR spectroscopy and OR measurements of the free phenols. The data were consistent with published values [11–14]. Compounds **4** and **7** were identified as their peracetates **4a** and **7a**, respectively. The ^{1}H NMR data for **4a** were in agreement with literature values [15]. The chemical shifts in the ^{1}H NMR spectrum of **7a** were consistent with published values [15] but ^{1}H–^{1}H long-range COSY led to a different assignment of the signals (Table 3). The major difference is the recognition of two rotamers in a ratio of 2:1 in our 400 MHz spectrum. ^{1}H–^{1}H long-range COSY generally detected the correlations between H-4 and H-6, H-8 and H-2, and also between H-2 and H-2′ and H-6′ of the same flavanol unit and thus allowed the assignment of all A- and C-ring protons of both rotamers.

Compound **8** exhibited a $[M+H]^+$ in the FAB-mass spectrum at *m/z* 867, indicating a trimeric procyanidin. Complete thiolysis yielded (−)-epicatechin-4β-benzylthioether (**2**) and (−)-epicatechin (**1**) as the only cleavage products. The lower interflavanoid bond was established as [4→8] by partial thiolysis and ^{1}H NMR identification of procyanidin B2 (**3**). As the ^{1}H NMR data of **8** were not identical with the spectrum of **7**, the upper linkage had to be [4→6]. Trimer **8** was thus identified as (−)-epicatechin-[4β→6]-epicatechin-[4β→8]-(−)-epicatechin, a compound isolated from *Kandelia candel* bark [16] and from Douglas fir (*Pseudotsuga menziesii*) inner bark [17].

The FAB-mass spectrum of compound **9** exhibited a $[M+H]^+$ peak at *m/z* 867 suggesting a trimeric procyanidin. Complete thiolysis yielded (−)-epicatechin-4β-benzylthioether (**2**) and (−)-epicatechin (**1**). The bonding positions were established by partial thiolysis and HPLC identification of procyanidin B5 (**4**) and (−)-epicatechin-[4β→8]-(−)-epicatechin-4β-benzylthioether (**5**) indicating a lower [4→6] linkage and an upper [4→8] linkage. Trimer **9** was thus identified as (−)-epicatechin-[4β→8]-(−)-epicatechin-[4β→6]-(−)-epicatechin, a compound previously isolated from *Rhaphiolepsis umbellata* bark [18].

Complete thiolysis of **10** yielded (−)-epicatechin-4β-benzylthioether (**2**) and (−)-epicatechin (**1**) in a ratio of 3:1 indicating a tetrameric structure. The bonding positions were established by partial thiolysis and HPLC identification of procyanidin C1 (**7**) and (−)-

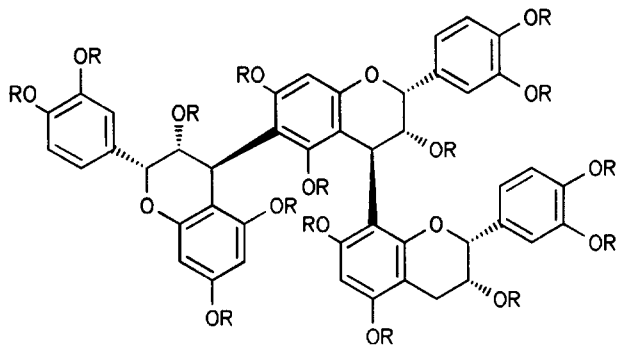
Table 3. ^{1}H NMR data of compound **7a** in $CDCl_3$ (400 MHz; standard $CHCl_3$ = 7.240 ppm) compared with the data published by Kolodziej [15] (*ma* = major rotamer, *mi* = minor rotamer; u = upper unit, m = middle unit, l = lower unit)

H	**7a** *ma* δ (J [Hz])	^{1}H–^{1}H long-range-COSY cross-peaks with	**7a** *mi* δ (J [Hz])	^{1}H–^{1}H long-range-COSY cross-peaks with	**7a** [15] δ (J [Hz])
2 u	5.39 *m*	3 u, 4 u, 2′ u, 6′ u	5.69 *br s*	3 u, 4 u, 2′ u, 6′ u	5.37 *m*
3 u	5.35 *m*	2 u, 4 u	4.95 *m*	2 u, 4 u	5.11 *m*
4 u	4.76 *br s*	2 u, 3 u, 6u, 8 u	4.48 *d* (2.0)	2 u, 3 u	4.66 *s*
6 u	6.64 *d* (2.25)	4 u, 8 u	6.24 *d* (2.25)	8 u	5.94 *d* (2.2)
8 u	6.75 *d* (2.25)	4 u, 6 u	5.93 *d* (2.25)	6 u	6.25 *d* (2.2)
2′ u	7.04–7.19 *m*	2 u, 6′ u	7.35 *d* (2.0)	2 u, 6′ u	7.15–7.34 *m*
5′ u	7.04–7.19 *m*	6′ u	7.04–7.28 *m*	6′ u	7.15–7.34 *m*
6′ u	7.04–7.19 *m*	2 u, 2′ u, 5′ u	7.04–7.28 *m*	2 u, 2′ u, 5′ u	7.15–7.34 *m*
2 m	5.35 *m*	3 m, 4 m, 2′ m, 6′ m	4.65 *br s*	3 m, 2′ m	4.76 *s*
3 m	5.39 *m*	2 m, 4 m	5.09 *br s*	2 m, 4 m	5.41 *m* or 5.47 *m*
4 m	4.69 *br s*	2 m, 3 m, 6 m	4.65 *br s*	3 m	4.69 *s*
6 m	6.64 *s*	4 m	6.88 *s* or 6.58 *s*		6.64 *s* or 6.69 *s*
2′ m	7.04–7.19 *m*	2 m, 6′ m	6.99 *d* (1.8)	2 m, 6′ m	7.15–7.34 *m*
5′ m	7.04–7.19 *m*	6′ m	6.93 *d* (8.25)	6′ m	7.15–7.34 *m*
6′ m	7.04–7.19 *m*	2 m, 2′ m, 5′ m	6.77 *dd* (1.8, 8.25)	2′ m, 5′ m	7.15–7.34 *m*
2 l	5.18 *br s*	3 l, 4 l α + β, 2′ l, 6′ l	5.10 *br s*	3 l, 4 l α + β, 2′ l	5.19 *s*
3 l	5.46 *m*	2 l, 4 l α + β	5.39 *m*	2 l, 4 l α + β	5.47 *m* or 5.41 *m*
4 l α	2.94 *br d* (18.0)	2 l, 3 l, 4 l β	2.88*	2 l, 3 l, 4 l β	3.00 *m*
4 l β	3.07 *dd* (5.0, 18.0)	2 l, 3 l, 4 l α	3.02*	2 l, 3 l, 4 l α	3.00 *m*
6 l	6.69 *s*		6.58 *s* or 6.88 *s*		6.69 *s* or 6.64 *s*
2′ l	7.28 *d* (1.8)	2 l, 6′ l	7.25†	2 l, 6′ l	7.15–7.34 *m*
5′ l	7.04–7.19 *m*	6′ l	7.04–7.28 *m*	6′ l	7.15–7.34 *m*
6′ l	7.04–7.19 *m*	2 l, 2′ l, 5′ l	7.04–7.28 *m*	2′ l, 5′ l	7.15–7.34 *m*
OAc	1.36–2.35 *m*		1.36–2.35 *m*		1.37–2.37 *m*

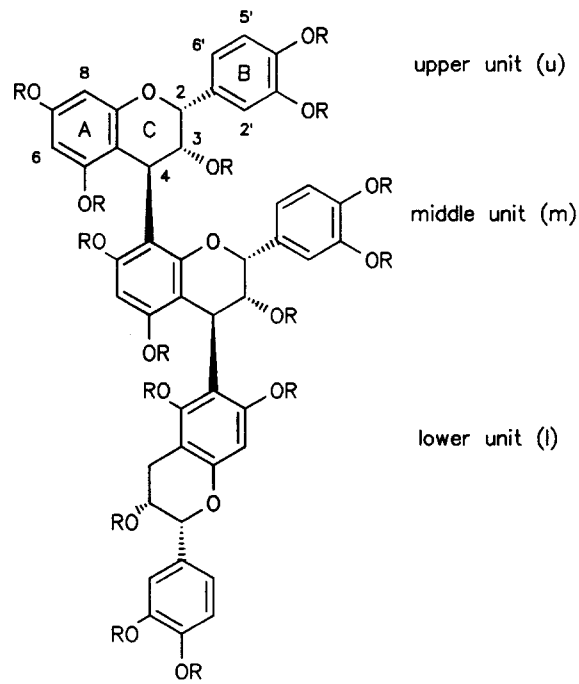
*Overlapping with *ma*.
†Overlapping with $CHCl_3$.

epicatechin - [4$\beta \rightarrow$8] - (−) - epicatechin - 4β - benzylthioether (**5**). With the formation of trimer **7** the lower two linkages were identified as [4 → 8]. As **5** was the only dimeric thioether formed by thiolysis, the upper linkage also had to be [4 → 8]. Tetramer **10** was thus identified as (−)-epicatechin-[4$\beta \rightarrow$8]-(−)-epicatechin - [4$\beta \rightarrow$8] - (−) - epicatechin - [4$\beta \rightarrow$8] - epicatechin, a compound previously isolated from *Cinnamomum cassia* bark [19].

For proanthocyanidin peracetates, shift parameters to distinguish between [4 → 8]- and [4 → 6]-linked dimers have been published. The upper A-ring signals of [4 → 8]-linked dimeric peracetates are shifted upfield to *ca* δ 6.1, whereas the upper A-ring protons of [4 → 6]-linked dimers resonate near δ 6.7 [20, 21]. In addition, H-2(l) of [4 → 8]-linked dimeric peracetates resonates between δ 4.37 and δ 5.01, whereas H-2(l) of [4 → 6]-linked dimers resonates between δ 5.04 and 5.35 [22]. To explain the upfield shifts of H-6(u), H-8(u) and H-2(l) of [4 → 8]-linked dimeric peracetates a conformation with the B-ring of the lower unit lying above the A-ring of the upper unit has been suggested [20]. The validity of these parameters has not been investigated systematically for trimeric and tetrameric peracetates. Therefore, compounds **7–10** were converted into their peracetates and analysed by ^{1}H NMR and ^{1}H–^{1}H long-range COSY. All but **8a** displayed rotational isomerism. The spectrum of **8a** consisted of only one set of signals. The two doublets of H-6(u) and H-8(u) were located at δ 6.57 and 6.65, respectively, corresponding well with the chemical shifts of the same protons of [4 → 6]-linked dimeric peracetates [21]. The chemical shift for H-2(m) of **8a** (δ 5.46) is also consistent with the H-2(l) chemical shift of [4 → 6]-linked dimers [22]. The chemical shifts for the upper A-ring protons at δ 5.93 and δ 6.24, and for H-2(m) at δ 4.65 of the minor rotamer of **7a** were in accordance with the chemical shifts of [4 → 8]-linked dimers



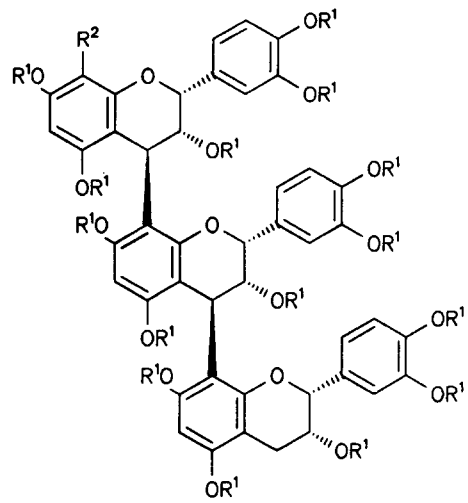
8: R = H
8a: R = Ac



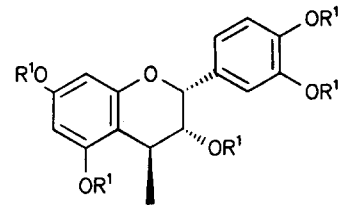
9: R = H
9a: R = Ac

[21, 22]. The spectrum of **9a** showed two major pairs of doublets for H-6(u) and H-8(u) with equal intensities. Probably, there were minor rotamers or other conformers present, as the spectrum showed further small A-ring signals and line-broadening in the heterocyclic region. One major pair of doublets resonated at δ 6.02 and 6.29, respectively, corresponding well with the chemical shifts of [4→8]-linked dimers [21]. Unfortunately, owing to poor resolution of the spectrum the assignment of the signals of H-2 and H-3 of the middle unit of **9a** was not possible. Therefore, the shift parameter for H-2 could not be verified for **9a**. The spectrum of **10a** was sharp and consisted of two sets of signals attributable to two rotamers in a ratio of 3:2. The A-ring protons of the minor rotamer resonated at δ 5.87 and 6.23, respectively. H-2 of the second upper unit of the minor rotamer was attributed to the broad singlet at δ 4.54. Thus, the validity of the shift rules for dimeric peracetates was also confirmed for the tetramer **10a**.

Beyond these known shift parameters a further remarkable feature of the minor rotamers of **7a** and **10a** was observed. H-2′, H-5′ and H-6′ of the second upper units were distinctly shifted upfield resonating between δ 6.67 and 6.99 (for individual chemical shifts see Table 3 and Experimental). These data are consistent with the conformation described above; not only are the upper A-ring protons in the shielding region of the



R^3:



7: $R^1 = R^2 = H$

7a: R^1 = Ac, $R^2 = H$

10: $R^1 = H$, $R^2 = R^3$

10a: R^1 = Ac, $R^2 = R^3$

second upper B-ring but the protons of the latter are also shielded by the upper A-ring. The spectrum of **9a** also showed signals between δ 6.80 and 6.98 which might be attributed to B-ring protons. However, unequivocal assignment of these signals was not possible owing to poor resolution and the lack of cross-peaks in the $^1H-^1H$ long-range COSY. Contrary to **7a**, **9a** and **10a**, all B-ring protons of **8a** resonated between δ 7.04 and 7.51. These findings suggest a new shift parameter to distinguish between oligomeric peracetates with an upper [4→8] linkage and oligomeric peracetates with an upper [4→6] interflavanoid bond and provide additional support for the suggested conformations.

Based on the conformation of dimeric peracetates as proposed by Fletcher *et al.* [20], and on the shift rules outlined above [21, 22], some general conclusions can be drawn concerning the conformation of oligomeric proanthocyanidin peracetates. In oligomers with an upper [4→8] linkage, rotational isomerism appears to occur mainly around the upper linkage, as for the two major rotamers only the shifts for the protons of the upper and second upper units differ considerably. The two major conformers which together account for *ca* 90% of the total substance may be described as follows. In one conformer, the B-ring of the second upper unit and the A-ring of the upper unit are lying one upon another, inducing a paramagnetic shielding effect on H-6 and H-8 of the upper unit and on H-2, H-2′, H-5′ and H-6′ of the second upper unit (Fig. 2A). The B-ring of the second upper unit of the other rotamer is situated in the opposite direction leading to the expected position of the signals of H-6(u) and H-8(u) and of H-2 and B-ring protons of the second upper unit (Fig. 2B). The remaining flavan-3-ol units of both rotamers are oriented with the B-rings directing away from the next upper unit. Contrary to dimers, in oligomeric peracetates with an upper [4→8] linkage the rotamer with the A-conformation is the minor rotamer or occurs, at best, in the same concentration as the B-conformer. In proanthocyanidin oligomers with the upper linkage [4→6] steric hindrance appears to be so strong that there is only one conformation.

EXPERIMENTAL

Plant material. For the preparation of the EtOH-extract (C), stem bark of a *ca* 15-year-old tree was harvested in March 1988 in Oaxaca, Mexico, and identified as *G. ulmifolia* Lam. (Sterculiaceae) by M. Heinrich A voucher specimen (no. Heinrich and Antonio B.: GUI 64) is deposited at the herbarium of the Institut für Pharmazeutische Biologie, Freiburg, Germany, and at the National Herbarium of Mexico (MEXU). For the Me_2CO percolate, stem bark of a *ca* 40-year-old tree was harvested in March 1994 in Oaxaca. A voucher specimen is deposited at the herbarium of the Institut für Pharmazeutische Biologie, Freiburg (Heinrich s/n, 1994).

General. ^1H NMR were recorded at 400 MHz; chemical shifts are given in δ (ppm). 2D NMR spectra ($^1H-^1H$ COSY and $^1H-^1H$-long-range (l.r.) COSY) were measured by the use of standard COSY pulse sequence and l.r.-COSY was optimized for coupling of

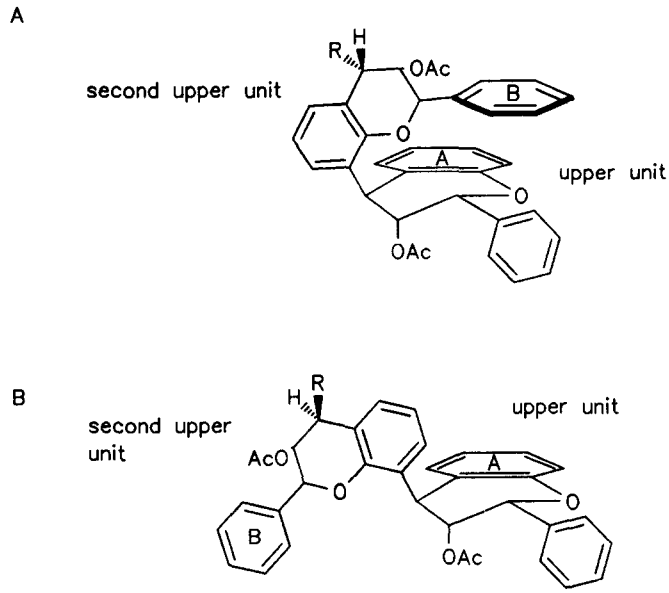


Fig. 2. Suggested conformations of oligomeric proanthrocyanidin peracetates (aromatic groups are omitted).

3 Hz. FAB-MS were obtained in the positive mode; matrix: glycerol–HOAc; acceleration 3 kV.

HPLC. Eurosphere C-18 column (5 μm, 250 × 4 mm, Knauer) protected with a guard cartridge packed with the same material. Detection: UV 280 nm. Mobile phase A: MeOH–MeCN–H_2O (5:4:1); mobile phase B: 0.02% TFA in H_2O.

CC. Sephadex LH-20, 25–100 μm (Pharmacia) and MCI-gel CHP-20P, 75–150 μm (Mitsubishi Chem. Ind.).

TLC. Silica gel 60 F_{254} (Merck); EtOAc–HCOOH–H_2O (18:1:1) (system A); detection vanillin–H_2SO_4 and $FeCl_3$. Cellulose (Merck); HOAc–HCl–H_2O (30:3:10) (Forestal); detection VIS.

Extraction and isolation. Air-dried and powdered bark (1040 g) was extracted with cold EtOH 70% (5 l, 3 min, Ultra turrax). After filtration, the bark was refluxed with EtOH 96% (5 l, 20 min) and EtOH 70% (5 l, 20 min, ×2). EtOH was removed *in vacuo* (40°) and the aq. residues of the hot and cold extracts combined and freeze-dried to yield 200 g crude extract (C). C (154 g) was dissolved in H_2O (2300 ml), washed with CH_2Cl_2 (3 × 2300 ml) and extracted with EtOAc (3 × 2300 ml, 1 × 1150 ml). After removal of solvents, the residues were lyophilized to yield 5.6 g CH_2Cl_2-layer (D), 11.9 g EtOAc-layer (E) and 132.8 g H_2O-layer (W). W (18 g) was chromatographed with EtOH 50% (5 l) on Sephadex LH-20 (column 440 × 37 mm). Frs were monitored by TLC in system A. The eluate was combined to 13 frs (W1.1–W1.13) of 100–300 ml at the beginning and 500–1000 ml at the end of CC. Frs W1.1 and W1.2 contained polysaccharides and W1.3–W1.13 contained oligomeric procyanidins. The remaining substances (6.2 g) were washed off the column with 2500 ml Me_2CO–H_2O (7:3) and further separated on Sephadex LH-20 with EtOH–H_2O–Me_2CO (9:9:2) (5100 ml, column 480 × 37 mm) to yield seven frs of 400–1000 ml (W2.1–W2.7). The remaining substances (3.3 g) were washed off the column with 2300 ml Me_2CO–H_2O (7:3) and were further chromatographed on Sephadex LH-20 with EtOH–H_2O–Me_2CO (7:7:6) (1800 ml, column 410 × 37 mm) to give six frs of 250–400 ml (W3.1–W3.6). The remaining substances (0.1 g) were washed off the column with 1500 ml Me_2CO–H_2O (7:3) (= W3.7). W3.1–W3.7 contained polymeric procyanidins.

Air-dried and powdered bark (111 g) was percolated in the dark at 10° with 1300 ml Me_2CO–H_2O (7:3) saturated with N_2. Me_2CO was removed *in vacuo* and the aq. residue freeze-dried to yield 21 g Me_2CO percolate (A). Liquid–liquid extraction of A (19 g) as described for the crude extract (C) gave 0.4 g CH_2Cl_2 layer (AD), 1.6 g EtOAc layer (AE) and 16 g H_2O layer (AW). AW was chromatographed on Sephadex LH-20 as described for W but all solvents were saturated with N_2. Frs AW3.1–AW3.6 contained polymeric procyanidins.

E was chromatographed on Sephadex LH-20 (580 × 34 mm) with EtOH 96% to yield 700 mg (−)-epicatechin (**1**) (920–1220 ml), 1120 mg procyanidin B2 (**3**) (1320–2000 ml) and 315 mg fr. E1.8 (2700–3300 ml). The remaining substances (2.14 g) were washed off the column with Me_2CO–H_2O 8:2 (= E2). E2 was further chromatographed on Sephadex LH-20 (580 × 34 mm) with EtOH 50% to yield 134 mg (−)-epicatechin-[4β→6]-(−)-epicatechin-[4β→8]-(−)-epicatechin (**8**) (2310–2625 ml) and 411 mg fr. E 2.6 (2755–3415 ml). Fr. E 1.8 was chromatographed on MCI-gel

(390 × 17.5 mm) with MeOH (35 → 45%, 5% steps); 16-ml frs were collected to give 102 mg procyanidin C1 (**7**) (frs 11–30) and fr. 78. Fr. 78 was further purified on MCI-gel (390 × 17.5 mm) with MeOH 50% (16-ml frs) to yield 27 mg procyanidin B5 (**4**) (frs 13–20). Fr. E 2.6 was chromatographed on MCI-gel (330 × 19 mm) with MeOH 25% 400 ml and MeOH (30 → 50%, 5% steps, 200 ml each); 20 ml frs were collected. Frs 48–51 yielded 49 mg (−)-epicatechin-[4β → 8]-(−)-epicatechin-[4β → 8]-(−)-epicatechin-[4β → 8]-(−)-epicatechin (**10**) and frs 57–60 contained 26 mg (−)-epicatechin-[4β → 8]-(−)-epicatechin-[4β → 6]-(−)-epicatechin (**9**). Note, in the following u = upper unit, um = upper middle unit, lm = lower middle unit, l = lower unit.

(−)-*Epicatechin* (**1**). $[\alpha]_D^{27}$ −30.9° (Me_2CO; *c* 1.18), ref. [12]: $[\alpha]_D$ −57.6° (Me_2CO; *c* 2.1). Difference may be due to unspecific impurities. ^1H NMR data consistent with published values [11].

Procyanidin B2 (**3**). $[\alpha]_D^{28}$ +31° (Me_2CO; *c* 0.9), ref. [14]: $[\alpha]_D^{25}$ +35.5° (Me_2CO; *c* 1.0). FAB-MS: *m/z* 579 $[M + H]^+$. ^1H NMR data consistent with published values [13]. Complete prep. thiolysis of **3** yielded **1** and **2**.

Procyanidin B5 (**4**). $[\alpha]_D^{27}$ +108° (Me_2CO; *c* 0.93), ref. for (+)-epicatechin-[4α → 6]-(+)-epicatechin [23]: $[\alpha]_D^{26}$ −105° (Me_2CO; *c* 0.993). ^1H NMR (Me_2CO-d_6, standard Me_2CO-d_5 = 2.04 ppm): δ 2.66 (1H, *dd*, *J* = 2.1, 16.5 Hz, H-4α(l)), 2.80 (1H, *dd*, *J* = 4.2, 16.5 Hz, H-4β(l)), 4.08 (1H, *br s*, H-3(u)), 4.17 (1H, *br s*, H-3 (l)), 4.66 (1H, *d*, *J* = 1.8 Hz, H-4(u)), 4.84 (1H, *br s*, H-2(l)), 4.98 (1H, *br s*, H-2(u)), 6.05 (1H, *s*, H-8(l)), 6.08 and 6.10 (1H each, *d*, *J* = 2.55 Hz, H-6(u) and H-8(u)), 6.73 (1H, *dd*, *J* = 1.8, 8.25 Hz, H-6′(u), 6.76 (1H, *d*, *J* = 8.25 Hz, H-5′(u)), 6.78 (1H, *d*, *J* = 8.25 Hz, H-5′(l)), 6.85 (1H, *dd*, *J* = 1.8, 8.25 Hz, H-6′(l)), 6.98 (1H, *d*, *J* = 18 Hz, H-2′(u)), 7.06 (1H, *d*, *J* = 1.8 Hz, H-2′(l)). Assignment of signals according to ^1H–^1H-l.r.-COSY. The 100 MHz ^1H NMR data of **4** in Me_2CO-d_6 [14] in agreement with our spectrum. ^1H NMR data of the peracetate of **4** (**4a**) were consistent with published values [15].

Procyanidin C1 (**7**). $[\alpha]_D^{27}$ +76.4° (Me_2CO; *c* 0.86), ref. [14]: $[\alpha]_D^{28}$ +75.2° (Me_2CO; *c* 0.87). FAB-MS: *m/z* 867 $[M + H]^+$. ^1H NMR (Me_2CO-d_6, standard Me_2CO-d_5 = 2.04 ppm): δ *ca* 2.7–2.8 (1H, overlapping with HDO, H-4α(l)), 2.93 (1H, *dd*, *J* = 5.4, 17.0 Hz, H-4β(l)), 4.07 (2H, *br s*, H-3(u), H-3(m)), 4.33 (1H, *br s*, H-3(l)), 4.80 and 4.82 (2H, 2 *br s*, H-4(u), H-4(m)), 5.06 and 5.15 (3H, 2 *br s*, H-2(u), H-2(m), H-2(l)), 5.96–6.03 (4H, *m*, H-6(u), H-8(u), H-6(m), H-6(l)), 6.68–6.80 (6H, *m*, H-5′(u), H-5′(m), H-5′(l), H-6′(u), H-6′(m), H-6′(l)), 6.95, 7.00 and 7.17 (1H each, 3 *br s*, H-2′(u), H-2′(m), H-2′(l)). 100 MHz ^1H NMR data of **7** in Me_2CO-d_6 [14] in agreement with our spectrum. Partial prep. thiolysis of **7** yielded **1**, **2**, **3** and **5**. ^1H NMR data of peracetate of **7** (**7a**) in Table 3.

(−) - *Epicatechin* - [4β → 6] - (−) - *epicatechin* - [4β → 8]-(−)-*epicatechin* (**8**). $[\alpha]_D^{26}$ +102.8° (Me_2CO; *c* 1.6), ref. [16]: $]\alpha]_D^{28}$ +138.0° (Me_2CO; *c* 1.0). Difference may be due to unspecific impurities. FAB-MS: *m/z* 867 $[M + H]^+$. ^1H NMR (Me_2CO-d_6, standard Me_2CO-d_5 = 2.04 ppm): δ 2.68 (1H, *br d*, H-4α(l)), *ca* 2.9 (1H, overlapping with HDO, H-4β(l)), 3.95 (1H, *m*, H-3(u) or H-3(m)), 3.98 (1H, *br s*, H-3(m) or H-3(u)), 4.26 (1H, *m*, H-3(l)), 4.58 (1H, *br s*, H-4(u) or H-4(m)), 4.68 (1H, *br s*, H-4(m) or H-4(u)), 4.93 (3H, *br s*, H-2(u), H-2(m), H-2(l)), 5.95–6.10 (4H, *m*, H-6(u), H-8(u), H-8 (m), H-6(l)), 6.65–6.86 (6H, H-5′(u), H-5′(m), H-5′(l), H-6′(u), H-6′(m), H-6′(l)), 6.98, 7.02 and 7.09 (1H each, 3 *br s*, H-2′(u), H-2′(m), H-2′(l)). 100 MHz ^1H NMR data of **8** in Me_2CO-d_6–D_2O [16] showed small differences from our data; these deviations can be attributed to the addition of D_2O and to the poor resolution of the 100 MHz spectrum. Complete analytical thiolysis yielded **2** and **1** in a ratio of 2:1. Partial prep. thiolysis of **8** yielded **1**, **2**, **3** and **6**. Acetylation yielded the peracetate **8a**. ^1H NMR ($CDCl_3$, standard $CHCl_3$ = 7.24 ppm): δ 1.25–2.38 (45H, 15 × OAc), 2.88–3.12 (2H, *m*, not resolved, H-4α(l), H-4β(l)), 4.39 (1H, *br s*, H-4(m)), 4.48 (1H, *d*, *J* = 1.6 Hz, H-4(u)), 4.94 (1H, *m*, H-3(u)), 5.14 (1H, *br s*, H-2(l)), 5.27 (1H, *m*, H-3(m)), 5.46 (2H, *br s*, H-2(m), H-3(l)), 5.67 (1H, *br s*, H-2(u)), 6.47 (1H, *s*, H-6(l)), 6.57 (1H, *d*, *J* = 2.25 Hz, H-6(u)), 6.65 (1H, *d*, *J* = 2.25 Hz, H-8(u)), 6.84 (1H, *s*, H-8(m)), 7.04–7.51 (9H, *m*, H-2′(u), H-2′(m), H-2′(l), H-5′(u), H-5′(m), H-5′(l), H-6′(u), H-6′(m), H-6′(l)).

(−) - *Epicatechin* - [4β → 8] - (−) - *epicatechin* - [4β → 6]-(−)-*epicatechin* (**9**). $[\alpha]_D^{26}$ +123.9° (Me_2CO; *c* 1.64), ref. [18]: $[\alpha]_D^{18}$ +126.8° (Me_2CO; *c* 1.15). FABMS: *m/z* 867 $[M + H]^+$. ^1H NMR (Me_2CO-d_6, standard Me_2CO-d_5 = 2.04 ppm): δ *ca* 2.7–2.9 (2H, overlapping with HDO, H-4 α(l), H-4β(l)), 4.09 (1H, *br s*, H-3(u) or H-3(m)), 4.19 (2H, *br s*, H-3(l) and H-3(u) or H-3(m)), 4.75 and 4.83 (1H each, 2*br s*, H-4(u), H-4(m)), 4.85 (1H, *s*, H-2(l)), 5.11 (2H, *br s*, H-2(u), H-2(m)), 5.98–6.07 (4H, *m*, H-6(u), H-8(u), H-6(m), H-8(l)), 6.72–6.85 (6H, *m*, H-5′(u), H-5′(m), H-5′(l), H-6′(u), H-6′(m), H-6′(l)), 6.99–7.07 (3H, *m*, H-2′(u), H-2′(m), H-2′(l)). 100 MHz ^1H NMR data of **9** in Me_2CO-d_6 [18] in agreement with our spectrum. A part of the spectrum is presented; the signals resolved are identical to our spectrum but because of line-broadening in the published spectrum an exact comparison was not possible. Complete analytical thiolysis yielded **2** and **1** in a ratio of 2:1. Partial analytical thiolysis yielded **1**, **2**, **4** and **5**. Acetylation yielded the peracetate **9a**. ^1H NMR ($CDCl_3$, standard $CHCl_3$ = 7.24 ppm, *A* = rotamer A, *B* = rotamer B, ratio 1:1): δ 1.23–2.35 (45H, 15 × OAc), 2.7–3.08 (2H, *m*, H-4α(l) *A* and *B*, H-4β(l) *A* and *B*), 4.05 (0.5H, *br s*, H-4(m) *A* or *B*), 4.34 (0.5 H, *br s*, H-4(m) *B* or *A*), 4.46 (0.5H, *d*, *J* = 2.4 Hz, H-4(u) *B*), 4.69 (0.5H, *br s*, H-4(u) *A*), 4.94 (0.5H, *m*, H-3(u) *B*), 5.12 (0.5H, *br s*, H-2(l) *A* or *B*), 5.16 (1H, *br s*, H-2(l) *B* or *A*), 5.22 (0.5H, *br s*, H-2(u) *A*), 5.49 (0.5H, *br s*, H-3(u) *A*), 5.66 (0.5H, *br s*, H-2(u) *B*), 6.02 (0.5H, *d*, *J* = 2.25 Hz, H-8(u) *B*), 6.29 (0.5H, *d*, *J* = 2.25 Hz, H-6(u) *B*), 6.62 (1H, *s*, H-6(m) *A* and H-6(m) *B* or H-8(l) *A* or H-8(l)

B), 6.65 (0.5 H, *s*, H-6(m) *B* or H-8(l) *A* or H-8(l) *B*), 6.68 (0.5H, *d*, $J = 2.25$ Hz, H-6(u) *A*), 6.73 (0.5H, *d*, $J = 2.25$ Hz, H-8(u) *A*), 6.76 (0.5H, *s*, H-6(m) *B* or H-8(l) *A* or H-8(l) *B*), 6.80–7.36 (9H, *m*, H-2′(u), H-2′(m), H-2′(l) *A* and *B*, H-5′(u), H-5′(m), H-5′(l) *A* and *B*, H-6′(u), H-6′(m), H-6′(l) *A* and *B*). Unequivocal assignment of H-2(m) *A* and *B* and of H-3(m) *A* and *B* not possible.

(−) - *Epicatechin* - [4$\beta \rightarrow$8] - (−) - *epicatechin* - [4$\beta \rightarrow$8] - (−) - *epicatechin* - [4$\beta \rightarrow$8] - (−) - *epicatechin* (**10**). $[\alpha]_D^{26}$ +109.5° (Me_2CO; *c* 1.23), ref. [19]: $[\alpha]_D^{23}$ +89.2° (Me_2CO; *c* 0.9). ^{1}H NMR (Me_2CO-d_6, standard Me_2CO-d_5 = 2.04 ppm): δ *ca* 2.7–2.8 (1H, overlapping with HDO, H-4α(l)), 2.94 (1H, *dd*, $J = 4.5$, 16.5 Hz, H-4β(l)), 4.08 (1H, *br s*) and 4.12 (2H, *br s*, H-3(u), H-3(um), H-3(lm)), 4.35 (1H, *br s*, H-3(l)), 4.83 (2H, *br s*) and 4.90 (1H, *br s*, H-4(u), H-4(um), H-4(lm)), 5.07 (1H, *br s*), 5.15 (1H, *br s*) and 5.29 (2H, *br s*, H-2(u), H-2(um), H-2(lm), H-2(l)), 5.96–6.03 (5H, *m*, H-6(u), H-8(u), H-6(um), H-6(lm), H-6(l)), 6.68–6.83 (8H, *m*, H-5′(u), H-5′(um), H-5′(lm), H-5′(l), H-6′(u), H-6′(um), H-6′(lm), H-6′(l)), 6.95, 6.99, 7.08 and 7.17 (1H each, 4*br s*, H-2′(u), H-2′(um), H-2′(lm), H-2′(l)). 100 MHz ^{1}H NMR data of **10** in Me_2CO-d_6 [19] in agreement with our spectrum. Complete analytical thiolysis yielded **2** and **1** in a ratio of 3:1. Partial analytical thiolysis yielded **1**, **2**, **3**, **5** and **7**. Acetylation yielded the peracetate **10a**. ^{1}H NMR ($CDCl_3$, standard $CHCl_3$ = 7.24 ppm, *ma* = major rotamer, *mi* = minor rotamer, ratio 3:2): δ 1.33–2.36 (60H, 20 × OAc *ma* and *mi*), 2.93 (0.4H, *br d*, $J = 18$ Hz, H-4α(l)*mi*), 2.94 (0.6H, *br d*, $J = 18$ Hz, H-4α(l)*ma*), 3.04 (0.4H, *dd*, $J = 4.5$, 18 Hz, H-4β(l)*mi*), 3.06 (0.6H, *dd*, $J = 4.6$, 18 Hz, H-4β(l)*ma*), 4.50 (0.4H, *d*, $J = 2.4$ Hz, H-4(u)*mi*), 4.54 (0.4H, *br s*, H-2(um)*mi*), 4.60 (0.4H, *br s*, H-4(lm)*mi*), 4.65 (0.6H, *br s*, H-4(lm)*ma*), 4.75 (0.6H, *br s*, H-4(u)*ma*), 4.78 (0.4H, *br s*, H-4(um)*mi*), 4.82 (0.6H, *br s*, H-4(um)*ma*), 4.95 (0.4H, *m*, H-3(u)*mi*), 5.13 (0.4H, *br s*, H-3(um)*mi*), 5.18 (1H, 2 *br s*, H-2(l)*ma* and *mi*), 5.26 (1H, *br s*, H-2(lm)*ma*, H-3(lm)*mi*), 5.29 (0.6H, *m*, H-3(u)*ma*), 5.31 (0.6H, *m*, H-3(lm)*ma*), 5.33 (1*H*, *br s*, H-3(um)*ma*, H-2(lm)*mi*), 5.42 (1.2H, *br s*, H-2(u)*ma*, H-2(um)*ma*), 5.46 (1H, *br s*, H-3(l)*ma* and *mi*), 5.72 (0.4 H, *br s*, H-2(u)*mi*), 5.87 (0.4H, *d*, $J = 2.25$ Hz, H-8(u)*mi*), 6.23 (0.4H, *d*, $J = 2.25$ Hz, H-6(u)*mi*), 6.57 (0.4H, *s*, H-6(l)*mi*), 6.60 (0.4H, *s*, H-6(lm)*mi*), 6.63 (0.6H, *s*, H-6(l)*ma*), 6.63 (0.6H, *d*, $J = 2.25$ Hz, H-6(u)*ma*), 6.67 (0.4H, *dd*, $J = 2.0$, 8.25 Hz, H-6′(um)*mi*), 6.69 (0.6H, *s*, H-6(lm)*ma*), 6.73 (0.6H, *s*, H-6(um)*ma*), 6.75 (0.6H, *d*, $J = 2.25$ Hz, H-8(u)*ma*), 6.87 (0.4H, *s*, H-6(um)*mi*), 6.91 (0.4H, *br s*, H-2′(um)*mi*) 6.92 (0.4H, *d*, $J = 8.25$ Hz, H-5′(um)*mi*), 6.95–7.34 (10.8H, *m*, H-2′(um)*ma*, H-2′(u), H-2′(lm), H-2′(l)*ma* and *mi*, H-5′(um)*ma*, H-5′(u), H-5′(lm), H-5′(l)*ma* and *mi*, H-6′(um)*ma*, H-6′(u), H-6′(lm), H-6′(l)*ma* and *mi*).

GPC. LKB Bromma HPLC-pump using a Knauer dual detector (RI and UV, 280 nm). Peracetylated proanthocyanidins were analysed on 10^3, 10^4, 10^5 and 10^6 Å PL-gel columns (300 × 7.7 mm; Polymer Lab.) connected in series. Elution was isocratic with $CHCl_3$ at 0.5, 0.75 and 1 ml min^{-1}, respectively. The system was calibrated with epicatechin peracetate (M_r 500), procyanidin B2 peracetate (M_r 998), procyanidin C1 peracetate (M_r 1496) and polystyrene standards (M_r 794, 2000, 4000, 10 300, 50 000 and 110 000). The calibration curve was generated using cubic splines.

$\overline{M_N}$ determination by complete thiolysis. Sample (3 mg) were dissolved in 300 μl EtOH 96%, 30 μl toluene-α-thiol and 15 μl HOAc were added under N_2. The sealed vial was kept for 120 hr at 94°. This mixt. was directly analysed by HPLC using the following elution conditions: flow rate 1 ml min^{-1}; mobile phase A, MeOH–MeCN–H_2O (5:4:1); mobile phase B, 0.02% TFA in H_2O; linear gradient from 30 to 70% A in 28 min, isocratic for 4 min, from 70 to 100% A in 2 min, followed by washing for 11 min and reconditioning of the column. Calibration was performed using (−) -epicatechin-4β-benzylthioether (obtained by complete thiolysis of **3**) and (−)-epicatechin (Fluka AG) as standards; R_t 28.0 and 8.8 min, respectively. Standard solns with molar ratios ((−)-epicatechin-4β-benzylthioether:(−)-epicatechin) of 28.7:1, 17.8:1, 10.9:1 and 1:1 were measured and calibration factors for the different ratios calculated. The calibration factor (equimolecular ratio of peak areas of epicatechin-4β-benzylthioether to epicatechin) varied between 1.02 for the 1:1-standard and 0.65 for the 28.7:1-standard. The calibration factor for a certain polymer fr. was selected depending on its GPC result. Values are means of three replicated injections.

Identification of extension units by complete thiolysis. The products of complete thiolysis of polymeric frs were identified by HPLC addition analysis with authentic samples. The only cleavage products were (+)-catechin (**11**) (R_t = 6.4 min), (−)-epicatechin (**1**) (R_t = 8.8 min), (+)-catechin-4β-benzylthioether (**12**) (R_t = 25.9 min) and (−)-epicatechin-4β-benzylthioether (**2**) (R_t = 28.0 min). The peak of (+) - catechin - 4α - benzylthioether (**13**) (R_t = 24.2 min) was too small to be detected unequivocally. Therefore, this cleavage product was neglected. Authentic samples: **1** from Fluka AG; **11** from Roth; **2** obtained by complete thiolysis of **3**; **12** and **13** obtained by complete thiolysis of proanthocyanidins from *Quercus petraea* bark [24]. During thioacidolysis, epimerization may occur [25]. Therefore, we determined the rate of conversion of **1**, **2** and **11**. Under our experimental conditions only **1** was epimerized to 2%. This rate was taken into account for estimation of the polymer composition.

Acetylation. Sample (25 mg) were dissolved in 1 ml pyridine and 1 ml Ac_2O. After stirring at room temp. for 48 hr, excess reagent was decomposed by addition of ice H_2O and the resulting ppt. collected by filtration.

Acid hydrolysis. W (1 mg) was dissolved in 0.2 ml *n*-BuOH–HCl (19:1) and 5 μl of a 2% (w/v) soln of ferric reagent ($(NH_4)Fe(SO_4)_2 \times 2H_2O$) in 2N HCl added. The mixt. was sealed in 1 ml glass vials and kept for 60 min at 100°. The soln was examined by TLC (Forestal), the pigment zone scraped off, eluted

and photometrically measured in 0.01% HCl–MeOH. W gave only one pigment with $R_f = 0.42$ and UV/VIS (0.01% HCl–MeOH) λ_{max} nm: 273, 536. These data were consistent with data obtained from an authentic sample for cyanidin-HCl and with lit. values [26].

Analytical, partial thiolysis. Partial thiolysis of fr. W3.3 and compounds **9** and **10** was performed as described for '$\overline{M_N}$ determination by complete thiolysis' but the reaction time was only 10 hr (5 hr for compound **10**) at 94°. Degradation products were identified by HPLC addition analysis using the same elution conditions as described above. R_t for the cleavage products **2**, **5**, **1**, **3** and **4** were 28.0, 23.8, 8.8, 6.4 and 13.6 min, respectively. Authentic samples: **1** from Fluka AG; **3**, **4** and **7** isolated from E and unequivocally identified; **2** complete thiolysis of **3**; **5** partial thiolysis of **7**. R_t of **6** (= 27.3 min) was determined by partial thiolysis of **8**. The chain-terminating flavan-3-ols of compound **10** were analysed using the following gradient: linear from 20% to 40% A in 25 min, isocratic for 5 min, linear gradient from 40% to 100% A in 3 min, followed by washing for 17 min and reconditioning of the column. The R_t for the cleavage products **3** and **7** were 14.0 and 17.5 min, respectively.

Partial or complete, preparative thiolysis. Samples (30 mg) were dissolved in 3 ml EtOH 96%, 150 μl toluene-α-thiol and 60 μl HOAc added under N_2. The vial was sealed and kept for 10–15 hr for partial thiolysis or 24 hr for complete thiolysis at 94°. After evapn of solvent, the oily residue was flash chromatographed on MCI-gel (30 × 10 mm) with MeOH (15% → 100%, 5% steps). Thiolysis of **3** yielded **2** and **1**, thiolysis of **7** yielded **5**, **2** and a mixt. of **1**, **3** and **7**, which were separated on Sephadex LH-20 (260 × 11 mm) with EtOH 96% as eluent. Thiolysis of **8** yielded a mixt. of the thioethers **2** and **6** and a mixt. of **1**, **3** and **8**. These mixts were separated on Sephadex LH-20 (260 × 11 mm) with EtOH 96% as eluent.

(−)-Epicatechin-4β-benzylthioether (**2**). $[\alpha]_D^{26}$ −9.6° (Me_2CO; *c* 1.147) (from thiolysis of W 3.1), lit. for (+) - epicatechin - 4α - benzylthioether [23]: $[\alpha]_D^{28}$ +29° (Me_2CO; *c* 0.31). HPLC showed that smaller amounts of **5** and **6** were also present which both have positive OR values. This explains the low value for **2**. ^{1}H NMR data consistent with published values [27].

(−) - Epicatechin - [4β → 8] - (−) - epicatechin - 4β - benzylthioether (**5**). ^{1}H NMR (Me_2CO-d_6, standard acetone Me_2CO-d_5 = 2.04 ppm): δ 3.98 (1H, *br s*, H-3(u)), 4.01 and 4.06 (1H each, AB, *J* = 13.5 Hz, –S–CH_2–), 4.07 (1H, *br s*, H-3(l)), 4.13 (1H, *br d*, *J* = 1.8 Hz, H-4(l)), 4.72 (1H, *br s*, H-4(u)), 5.12 (1H, *br s*, H-2(u)), 5.32 (1H, *br s*, H-2(l)), 5.95–6.01 (3H, *br s*, H-6(u), H-8(u), H-6(l)), 6.70–6.83 (4H, *m*, H-5′(u), H-5′(l), H-6′(u), H-6′(l)), 6.96 (1H, *br s*, H-2′(u)), 7.05 (1H, *br s*, H-2′(l)), 7.23 (1H, *m*, H-4 benzyl-ring), 7.31 (2H, *m*, H-3 and H-5 benzyl-ring), 7.46 (2H, *m*, H-2 and H-6 benzyl-ring). 100 MHz ^{1}H NMR data of **5** in Me_2CO-d_6–D_2O [16] showed small differences from our data. These deviations can be attributed to the addition of D_2O and to the poorer resolution of the 100 MHz spectrum.

(−) - Epicatechin - [4β → 6] - (−) - epicatechin - 4β - benzylthioether (**6**). ^{1}H NMR (Me_2CO-d_6, standard Me_2CO-d_5 = 2.05 ppm): δ 3.99 (1H, *m*, H-3(l)), 3.98–4.06 (2H, not resolved, –S–CH_2–), 4.05 (1H, *d*, *J* = 2 Hz, H-4(l)), 4.13 (1H, *m*, H-3(u)), 4.67 (1H, *d*, *J* = 1.6 Hz, H-4(u)), 5.03 (1H, *br s*, H-2(u)), 5.23 (1H, *br s*, H-2(l)), 6.05 (1H, *br s*, H-8(l)), 6.09–6.11 (2H, not resolved, H-6(u), H-8(u)), 6.70–6.84 (4H, *m*, H-5′(u), H-5′(l), H-6′(u), H-6′(l)), 6.98 and 7.05 (1H each, H-2′(u), H-2′(l)), 7.20–7.52 (5H, *m*, benzyl-ring). 100 MHz ^{1}H NMR data of **6** in Me_2CO-d_6–D_2O [16] showed small differences from our data. These deviations can be attributed to the addition of D_2O.

SDS–PAGE. Cholera toxin (8 μg) dissolved in 20 μl H_2O was treated for 15 min with the test samples dissolved in 10 μl H_2O. Sample buffer (30 μl, 3.2 ml 0.5M Tris–HCl pH 6.8; 2.3 g glycerol 87%; 4.0 ml SDS 10%; 0.5 ml Bromphenol Blue 0.4%) and 5 μl 2-mercaptoethanol were added and the mixt. kept for 7 min at 100°. Denaturated proteins were analysed by SDS–PAGE according to ref. [28] and stained with Coomassie-Blue. The lowest dose (μg) at which no A-band of the toxin was detectable was determined for frs W3.1 to W3.7. The results were as follows: W3.1: 7.5; W3.2: 15; W3.3: 30; W3.4: 15; W3.5: 15; W3.6: 15; W3.7: 15.

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