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POLYPHENOLIC COMPOUNDS FROM *CROTON LECHLERI**

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Key Word Index—*Croton lechleri*; Euphorbiaceae; Sangre de Grado; Dragon's blood; polyphenol; proanthocyanidins; flavan-3-ols; procyanidins; prodelphinidins.

Abstract—The blood-red sap of *Croton lechleri* was found to contain proanthocyanidins as major constituents which accounted for up to 90% of the dried weight. In addition to (+)-catechin, (−)-epicatechin, (+)-gallocatechin, (−)-epigallocatechin and dimeric procyanidins B-1 and B-4, five novel dimers and trimers were isolated and characterized as catechin-(4α→8)-epigallocatechin, gallocatechin-(4α→8)-epicatechin, gallocatechin-(4α→6)-epigallocatechin, catechin-(4α→8)-gallocatechin-(4α→8)-gallocatechin and gallocatechin-(4α→8)-gallocatechin-(4α→8)-epigallocatechin. Higher oligomers were also obtained. A new procedure combining chemical degradation with ^{1}H NMR spectroscopy has been developed for determination of the composition and molecular size of oligomeric/polymeric proanthocyanidins. The oligomers of the sap were shown to have the mean degree of polymerization of 4,5-6 and 6-7, respectively, and M_r up to 2130. The heterogeneity of the oligomers was clearly indicated by the presence of a variety of flavan-3-ols as extension and terminal units. An exceptionally high content of gallocatechin and epigallocatechin in the oligomers was observed.

INTRODUCTION

The bark of *Croton lechleri* L., when slashed, produces a blood-red sap, commonly termed Sangre de Grado or dragon's blood. This viscous latex has been used by South Americans for the treatment of a number of illnesses and diseases, such as wounds, cancer and rheumatism [1–3]. Medicinal significance and rapidly declining sources have aroused scientific interest in this plant. An alkaloidal compound, called taspine has been isolated from the sap of *Croton lechleri* and characterized [3]; it was shown to be the active wound healing principle [4]. A new dihydrobenzofuran lignan compound has been reported recently [5]. In the present communication a spectrum of proanthocyanidins from monomers to heptamers was isolated and characterized.

RESULTS AND DISCUSSION

The latex of *Croton lechleri* was extracted with chloroform and then ethyl acetate. A large volume of acetone was added to the residual aqueous solution to give an acetone-soluble fraction, and a methanol-soluble fraction was obtained from the acetone insoluble materials. Paper chromatography suggested that proanthocyanidins were the major constituents of the ethyl acetate, acetone and methanol extracts. The ethyl acetate extract was fractionated on Sephadex LH-20 and elution with ethanol gave fractions I and II, in which flavan-3-ol monomers and dimers were included, respectively. Further elution with ethanol–acetone (19:1) gave fractions III and IV which contained trimeric and tetrameric proanthocyanidins respectively. Higher oligomers were found to be present in the acetone- and methanol-soluble fractions V and VI.

Purification of fraction I was achieved on Sephadex LH-20 using chloroform–ethanol (7:3) as eluent, and compounds **1**–**4** were obtained. They were characterized by comparison to literature values using mass, ^{1}H and ^{13}C NMR spectroscopy to be (+)-catechin (**1**), (−)-epicatechin (**2**), (+)-gallocatechin (**3**) and (−)-epigallocatechin (**4**). The elution pattern from Sephadex LH-20 followed the order **2**→**1**→**4**→**3**.

Re-chromatography of fraction II on Sephadex LH-20 gave compounds **5**–**9**. The mass spectra (FAB-MS) of **5** and **6** exhibited $[M+H]^+$ peaks at m/z 579, which suggested that both compounds possess a structural skeleton of a B-type procyanidin dimer. Their ^{1}H NMR spectra displayed complex patterns due to the existence of conformers generated by the restricted interflavan rotation. At least two conformational isomers were indicated from the ^{1}H NMR spectrum of **6**, whilst that of **5** suggested that one conformer was predominant. Their distinctive H-2 and H-4 signals revealed the presence of catechin (**1**) and epicatechin (**2**) in both compounds, and of **1** and **2** as a lower unit in **5** and **6**, respectively. The ^{1}H NMR spectra of **5** and **6** therefore resembled those of procyanidins B-1 and B-4 [6]. On thiolytic degradation, **5** produced epicatechin 4-benzylthioether (**10**) and **1** and **6** yielded catechin 4-benzylthioether (**11**) and **2**. These monomeric products were identified by ^{1}H NMR spectra. Compounds **5** and **6** were thus characterized as procyanidins B-1 [epicatechin-(4β→8)-catechin] and B-4 [catechin-(4α→8)-epicatechin].

Compounds **7** and **8** were simultaneously eluted off the Sephadex LH-20 column. They occurred as a single spot

*Part 1 in the series, Biological and Chemical Investigation of Dragon's Blood from *Croton* Species of South America.

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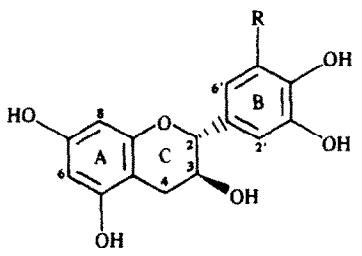

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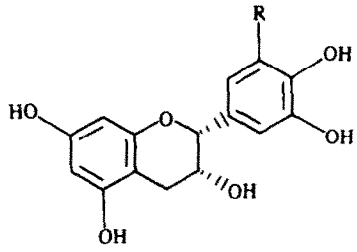

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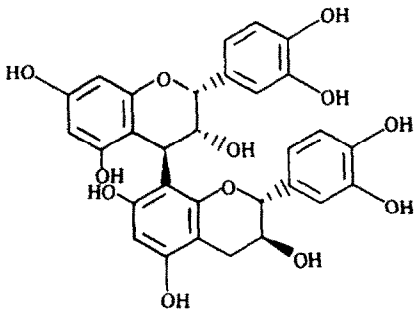

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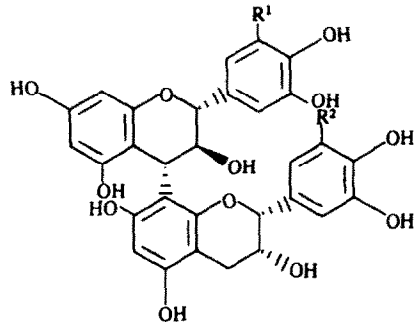
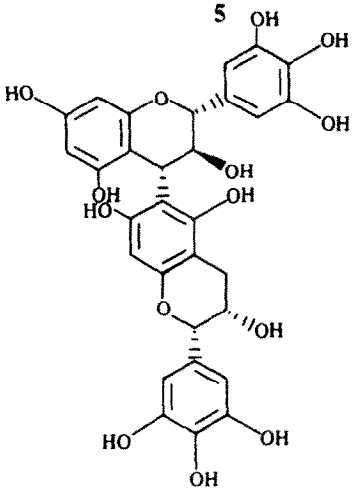


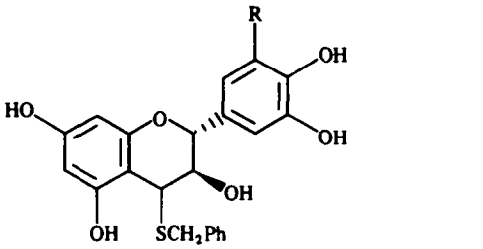
6 $R^1 = R^2 = H$
7 $R^1 = H$, $R^2 = OH$
8 $R^1 = OH$, $R^2 = H$



9

on paper chromatography [R_f (A), 0.42, R_f (B) 0.29]. Their separation was achieved by means of reverse-phase HPLC, the elution order being 7→8. The FAB mass spectral analysis demonstrated that both compounds exhibited identical $[M+H]^+$ and $[M+Na]^+$ ions at *m/z* 595 and 617, indicative of dimers consisting of catechin/epicatechin and gallocatechin/epigallocatechin. The examination of the characteristic ^1H signals for H-2, H-4 and the protons on the aromatic ring B indicated the existence of catechin (**1**) as upper unit and epigallocatechin (**4**) as lower unit in **7**, and of gallocatechin (**3**) as upper unit and epicatechin (**2**) as lower unit in **8**, despite the complexity of the spectra. The treatment of **7** with toluene-α-thiol in acidic media gave **11** and **4**, and gallocatechin 4-benzylthioether (**12**) and **2** were obtained by thiolytic degradation of **8**.

Determination of the absolute stereochemistry at C-4 of the upper unit of procyanidin dimers has been successfully achieved by Haslam [7]. This procedure utilizes the 'γ-effect' observed in the ^{13}C NMR spectra of the heterocyclic ring of the flavan-3-ol system as a probe. When an aromatic ring at C-4 adopts a *trans* orientation relative to the proton at C-2, it has little effect on the C-2 resonance. However, an aromatic ring at C-4 with a *cis* orientation induces a characteristic upfield shift (4.5 ppm) on the chemical shift of C-2 (Fig. 1). It was observed that the ^{13}C signals for the two C-2 of **7** and **8** occurred at δ83.4 and 79.6, and 83.8 and 79.0, quite similar to those of unsubstituted flavan-3-ols. No marked upfield shift for C-2 of the upper units was observed in either case. Therefore the lower units of **7** and **8** adopt the same orientation as observed in **6**, i.e. the α-orientation. This assignment was supported by the observed large coupling constant ($J=9.6$ Hz) for the H-4 of the upper unit of **7** and **8**. A much smaller $J_{3,4}$ value would be expected if the β-orientation is assumed by the lower unit. The position of



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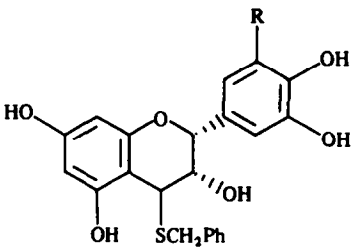
12 R = OH



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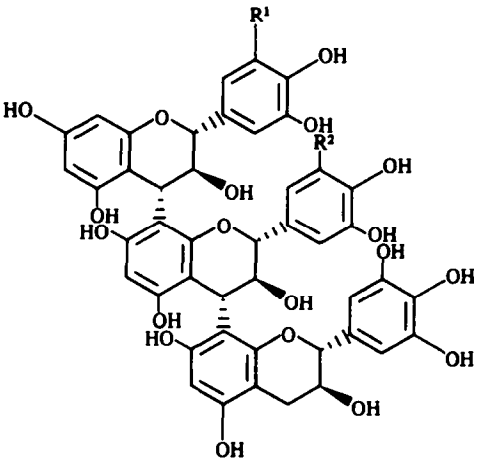
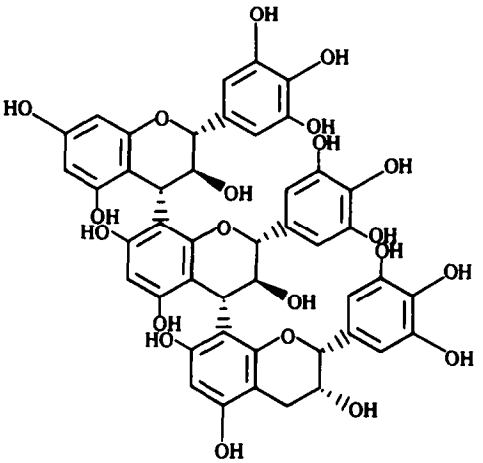
16 R = OH



13 R^1 = H or OH
R^2 = OH or H



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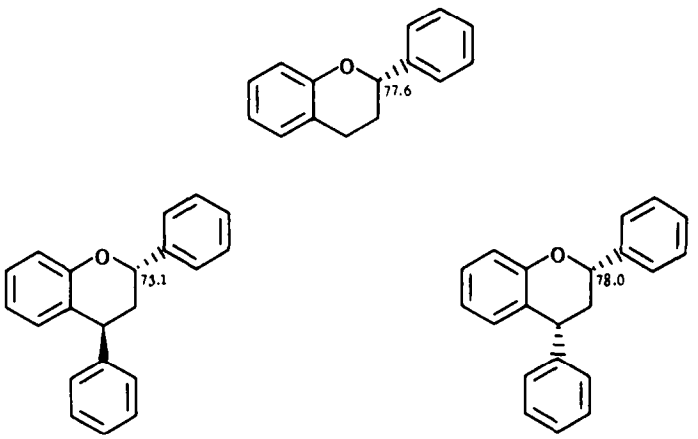


Fig. 1. ^{13}C NMR chemical shift (ppm): 'γ-effect'.

the interflavan linkage was deduced from their ^{1}H NMR data. The chemical shifts (δ4.91 and 5.02) for the lower H-2 signals were analogous to the corresponding ones (δ4.89 and 5.02) of 4α→8 linked procyanidin B-4 [8]. The position of the lower H-2 signals (δ4.84 and 4.89) of **8** was also close to those of B-4. These analyses led to the establishment of the structures for **7** and **8** as the novel compounds catechin-(4α→8)-epigallocatechin and gallocatechin-(4α→8)-epicatechin, respectively.

Compound **9** was repurified using reverse-phase HPLC. The FAB mass spectrum of **9** exhibited its $[M+H]^+$ and $[M+Na]^+$ peaks at *m/z* 611 and 633, respectively, corresponding to a prodelphinidin dimer. The ^{1}H NMR spectrum clearly revealed that it consisted of gallocatechin as upper unit and epigallocatechin as lower unit (Fig. 2). The large coupling constant (δ4.34, $J_{3,4}$ = 9.5 Hz) established the stereochemistry at C-4 of the upper unit, with the lower unit adopting the α-

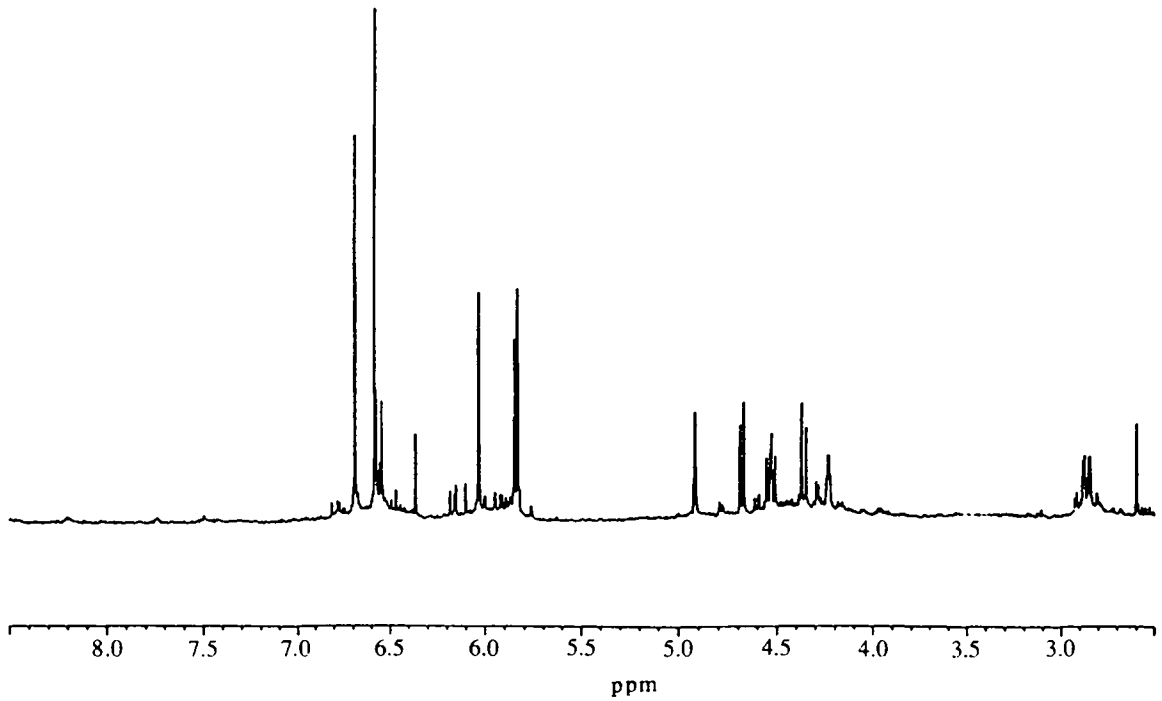


Fig. 2. ^{1}H NMR spectrum of compound **9** in d_6-acetone containing trace of D_2O (d_6-acetone as internal reference: δ2.05 ppm).

orientation. This was supported by the ^{13}C NMR spectrum which exhibited the occurrence of the upper C-2 resonance of δ83.3, similar to that of unsubstituted gallocatechin.

It is of interest to note that in the ^{1}H NMR spectrum of **9** (Fig. 2), one conformer is predominant (~90%), and the chemical shifts for H-2 and H-3 of the lower unit are similar to those of epigallocatechin (**4**). Marked differences between the ^{1}H NMR spectra of **9** and gallocatechin-(4α→8)-epigallocatechin as dodecacetates were observed, the latter being reported to be generally analogous to that of procyanidin B-4 [9]. Compared to the 4α-8 linked dimer, the dimers of 4β-8 and 4-6 linkages generally display largely simplified ^{1}H NMR spectra [6, 10]. Molecular models also suggest that the rotational restriction imposed on two units of 4-6 and 4β-8 linked dimers may be released to some extent. Accordingly the units in **9** are joined via a 4α-6 linkage, and hence **9** is the novel compound, gallocatechin-(4α→6)-epigallocatechin.

Two novel trimeric proanthocyanidins, **13** and **14**, were obtained from fraction III using reverse-phase HPLC. In the FAB mass spectrum, **13** exhibited $[M+H]^+$ and $[M+Na]^+$ ions at *m/z* 899 and 921, indicative of a trimer consisting of one catechin/epicatechin unit and two gallocatechin/epigallocatechin units. The complex ^{1}H NMR spectrum of **13** conveyed little structural information except for the presence of catechin/gallocatechin as a terminal unit indicated by two distinctive quartets at δ2.50 and 3.00. On thiolytic degradation **13** gave **11**, **12** and gallocatechin. In the ^{13}C NMR spectrum of **13** a broad signal (δ82.8–84.2, 3C) due to C-2 was observed, and there was no signal in the region of δ82.0–76.0. This led to the characterization of the stereochemistry at C-4 of the upper and middle units, i.e. the β-orientation was adopted. The great complexity of the ^{1}H NMR spectrum of **13** suggests that the three flavan-3-ol units are C-4/C-8 linked. The most probable structure is then represented as **13**.

The FAB mass spectrum of **14** exhibited $[M+H]^+$ and $[M+Na]^+$ ions at *m/z* 915 and 937, consistent with a trimeric prodelphinidin structure. Epigallocatechin (**4**) was identified as a terminal unit by its characteristic ^{1}H NMR signals for H-4. The ^{1}H NMR spectrum also indicated the occurrence of gallocatechin (a doublet at δ4.76, J = 7.7 Hz). Acid-catalysed thiolytic degradation of **14** yielded **12** and **4**, approximately in the ratio of 2:1. Two broad peaks around δ83.0 (2C) and 79.0 (1C) in its ^{13}C NMR spectrum suggested that the two interflavan linkages are α-orientated. The three flavan-3-ol units are therefore linked through C-4α/C-8 because **14** displayed a complex pattern in its ^{1}H NMR spectrum. Compound **14** was thus characterized as gallocatechin-(4α→8)-gallocatechin-(4α→8)-epigallocatechin.

Fraction IV produced a single spot [R_f (A) 0.21, R_f (B) 0.10] on paper chromatography, of lower R_f than previous fractions. Four peaks were discerned by reverse-phase HPLC analysis. However, further efforts to separate this mixture were not successful. This fraction was subjected to FAB-MS analysis, four pairs of $[M+H]^+$ and $[M+Na]^+$ peaks at *m/z* 1171, 1193; 1187, 1209; 1203, 1225; and 1219, 1241 were readily recognized and were of a higher intensity compared to the rest of the spectrum. These peaks corresponded to tetrameric proanthocyanidins with an increasing degree of hydroxylation on the aromatic ring B. The ^{1}H and ^{13}C NMR spectra of the tetramers consisted of a number of broad signals from which it was not possible to extract detailed structural information.

The low mobility of the acetone- and methanol-soluble fractions V and VI on paper chromatography suggested the occurrence of higher oligomeric proanthocyanidins. The heterogeneity of such oligomers was demonstrated by multiple peaks on HPLC. In these cases, FAB mass and NMR spectroscopy were shown to be of little value for their structural elucidation.

The structural features of oligomeric/polymeric proanthocyanidins have been investigated over the last two decades [11–13]. The results show that there is a diversity of flavan-3-ols as extension and terminal units, with different stereochemistry at C-4 of the heterocycles of each extension unit, coexistence of a number of types of interflavan linkages, (e.g. C-4/C-6 and C-4/C-8), varying degree of restricted rotation around each interflavan linkage and variable lengths of proanthocyanidin chains. Obviously, they will impose serious problems in the purification and structural elucidation of oligomeric/polymeric proanthocyanidins.

Attempts have been made to investigate in more detail the structures of proanthocyanidins, and one of the procedures developed by Haslam is based upon the HPLC analysis of the products of the acid-catalysed thiolytic degradation of proanthocyanidins [14]. By measurement using HPLC of the relative amounts of various flavan-3-ols (from terminal units) and their thioether derivatives (from extension units), the composition of the proanthocyanidins may be determined, and the average M_r estimated.

Another method utilizes ^{13}C NMR spectroscopy [15]. From the intensity of the characteristic ^{13}C resonance of flavan-3-ol components of intact oligomers/polymers, the composition is deduced and the average M_r derived. The application of this method has been recently extended to examine the stereochemistry of the heterocycles and the position of the interflavan linkages [16]. However, in our experience, the ^{13}C NMR signals (observed at 100.62 MHz) for oligomeric proanthocyanidins are generally broad, and some of these signals are frequently superimposed. Hence it is not necessarily easy to make proper assignments.

An alternative procedure has been developed in this laboratory, which combines chemical degradation with ^1H NMR spectroscopy, and provides a rapid and accurate analysis for proanthocyanidins. It was observed that the H-2 resonances of a variety of flavan-3-ols and their benzylthioether derivatives occur at different frequencies (Table 1), and the well separated signals permit an unambiguous assignment and accurate measurement of their relative amounts to be made.

The oligomeric proanthocyanidins obtained in the present work were analysed using this method. Fractions IV–VI were treated with toluene-α-thiol in acidic media. The reaction was maintained for 48 hr to allow a full decomposition to take place. After removal of the excessive thiol, the products were obtained from a silica gel column using chloroform–methanol (3:1) as eluent. They were then analysed as a mixture on a high frequency FT-NMR spectrometer (400 MHz). The H-2 signals of the products—flavan-3-ols and their thioether derivatives were satisfactorily separated and identified, and as a typical example, the ^1H NMR spectrum of the thiolytic products of the oligomers in fraction VI is presented in Fig. 3. Accurate measurements of the intensity of these signals were then made. Consequently, the relative amounts of the products were determined and the average M_r estimated. The details of the analysis for the oligomers in fractions IV–VI are shown in Table 2.

It was observed that the ratio of the terminal units to the extension units involved in the oligomers of fraction IV, as measured using this procedure was 1:2.9, confirming their tetrameric constitution. This was in agreement with their FAB mass spectrum. The heterogeneity of their structures was reflected in the presence of a variety of flavan-3-ols as extension and terminal units. The oligomers in fractions V and VI were shown to have a ratio of terminal to extension units of ratio of 1:4.4 and 1:5.3, respectively. Their mean degree of polymerisation was 5-6 and 6-7, and the average M_r was 1830 and 2130, respectively. Their structures may be represented by **17** and **18**.

The thiolytic products of two trimers (**13** and **14**) obtained from fraction III were also quantitatively analysed using this procedure. Catechin 4-benzylthioether (**11**), gallocatechin 4-benzylthioether (**12**) and gallocatechin (**3**) released by **13** were found in the ratio of 1.1:1.1:1; and **14** gave **12** and **4** in the ratio of 2.1:1. Hence, this procedure was further shown to be reliable.

The characterisation of the stereochemistry at C-4 and the position of the interflavan linkage in oligomeric proanthocyanidins remains difficult. The observation on the dimers and trimers obtained in the present work suggests that the α-configuration is favoured and C-4/C-8 and C-4/C-6 linkages co-exist. However, this remains to be clarified for the oligomers.

The oligomers in fractions IV–VI possessed an exceptionally high content of gallocatechin and epigallocatechin (up to 92%), whereas the majority of the oligomeric and polymeric proanthocyanidins found so far have a higher content of catechin and epicatechin. Accordingly, they may be classified into the category of prodelphinidins rather than procyanidins. This study shows that the latex of *Croton lechleri* is a rich source of soluble proanthocyanidins which account for up to 90% of the dried weight.

The blood-red saps from a number of *Croton* species are used as traditional medicines in South America [1–3] and the identification of plant material used may be difficult to establish. In addition to *Croton lechleri* (Sangre de Grado, dragon's blood), *C. draconoides* (Muell.) Arg. (Sangre de Drago) is similarly used. Other red sap containing species which are used medicinally

Table 1. Characteristic ^1H NMR signals for H-2 of flavan-3-ols and their thioether derivatives in d_6-acetone*

Compound	H-2 (δppm)†
(+)-Catechin (**1**)	4.55 *d* (6.7)
(−)-Epicatechin (**2**)	4.88 *s*
(+)-Gallocatechin (**3**)	4.51 *d* (7.3)
(−)-Epigallocatechin (**4**)	4.82 *s*
Catechin 4-benzylthioether (**11**)	4.91 *d* (9.7)
Epicatechin 4-benzylthioether (**10**)	5.30 s
Gallocatechin 4-benzylthioether (**12**)	4.89 *d* (9.6)
Epigallocatechin 4-benzylthioether (**16**)	5.21 *s*

*d_6-Acetone as internal reference: δ2.05.

†Slight upfield shift of these signals was observed when present in a mixture, as seen in Fig. 3.

J (Hz) in parentheses.

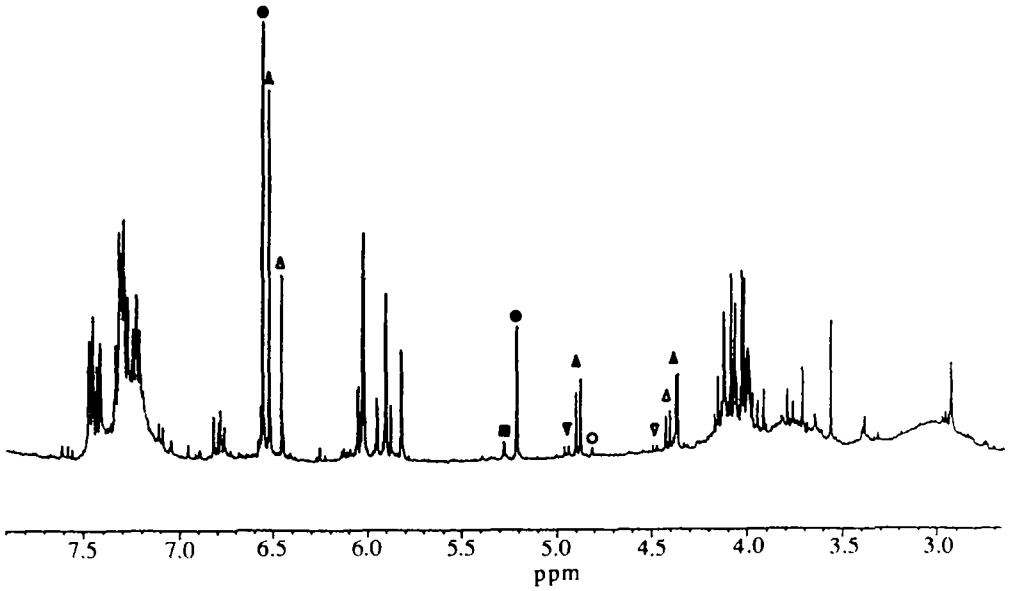


Fig. 3. Characteristic signals in the ^{1}H NMR spectrum of the thiolytic products of the oligomers in fraction VI in d_6-acetone. ● Epigallocatechin 4-benzylthioether (**16**), ○ epigallocatechin (**4**), ■ epicatechin 4-benzylthioether (**10**), ▽ catechin (**1**), ▼ catechin 4-benzylthioether (**11**), △ gallocatechin (**3**), ▲ gallocatechin 4-benzylthioether (**12**).

Table 2. Relative amounts of flavan-3-ols and their thioether derivatives in the oligomers, determined using ^{1}H NMR spectroscopy in d_6-acetone

Fraction	Extension unit (E)*				Terminal unit (T)*				Ratio of E to T
	CAT (**11**)	ECT (**10**)	GCT (**12**)	EGCT (**16**)	CA (**1**)	EC (**2**)	GC (**3**)†	ECG (**4**)	
IV	4.6	1.7	31.8	1.9	1.5	1.3	10.0	1.1	1.9:1
V	3.2	3.6	44.4	19.3	0.0	6.1	10.0	0.0	4.4:1
VI	1.7	2.0	29.6	31.3	1.1	1.1	10.0	0.0	5.3:1

*CA, EC, GC and EGC are the abbreviations for catechin, epicatechin, gallocatechin, epigallocatechin and CAT, ECT, GCT, EGCT are the abbreviations for their thioether derivatives, respectively.

†For convenience of comparison, the amount of GC (**3**) was fixed at 10.0.

include *C. draco* Schlect (Mexico), *C. echinocarpus* (Muell.) Arg. (Brazil), *C. gossipifolius* (Venezuela, Mexico), *C. hibiscifolius* (Argentina), *C. palanostigma* Klotsch (Peru, Brazil), *C. urucurana* Baill. (Paraguay) and *C. xalapensis* H.B.K. (Guatemala, Mexico). In the present communication the sap has been obtained from *C. lechleri*.

EXPERIMENTAL

^{1}H (400 MHz) and ^{13}C (100.62 MHz) NMR spectra. FAB-MS: thioglycerol was used as matrix. Reverse-phase HPLC was conducted on Altex 110A HPLC system, column: Altex Ultrasphere-ODS (1 × 25 cm); eluent: 10% aq. MeOH; flow rate: 2 ml min^{-1}. UV detector: 280 nm. Paper chromatography (Whatman No. 2 paper) was developed in the solvent system: (A) 6% aq. HOAc; and (B) butan-2-ol–HOAc–H_2O (14:1:5), and compounds were located using a fresh mixt. of 1% $FeCl_3$ and 1% $K_3Fe(CN)_6$ aq. solns.

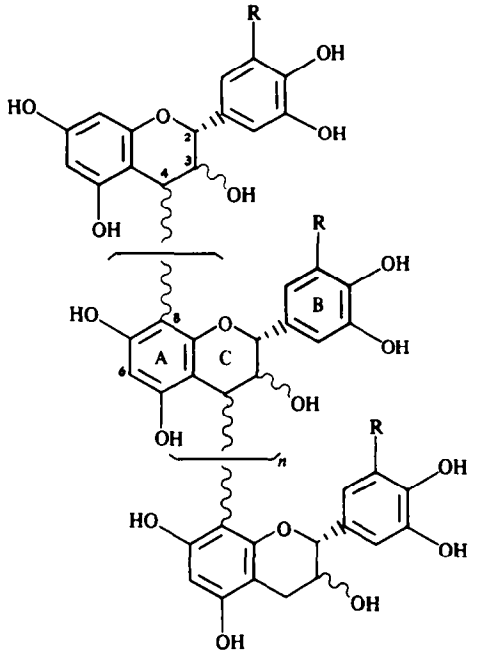
Sample. The blood red latex of *Croton lechleri* (Euphorbiaceae) was collected in Ecuador by two of us (M.H.Z., Y.Y.G.).

Isolation. The latex (100 ml) was extracted successively with $CHCl_3$ (3 × 250 ml) and EtOAc (6 × 250 ml) to give 2 frs (0.25 and 4.50 g, respectively). Me_2CO (500 ml) was then added to the residual aq. soln to give an Me_2CO-soluble fr. (11.20 g). The insoluble materials were dissolved in MeOH (500 ml), and removal of the residues gave a MeOH-sol. fr. (4.70 g).

The EtOAc extract (3.0 g) was chromatographed on a Sephadex LH-20 column (3 × 70 cm, in EtOH). The elution was initiated with EtOH (1.2 l) to give frs I (0.82 g) and II (1.01 g). A mixt. of EtOH and Me_2CO (19:1) was then used as eluent to give frs III (0.17 g) and IV (0.12 g). Throughout the separation process, the fractionation was monitored by paper chromatography.

Isolation and identification of flavan-3-ols in fraction I. The flavan-3-ol monomers (0.82 g) were rechromatographed on Sephadex LH-20 (1 × 50 cm) using $CHCl_3$–EtOH (7:1) as eluent, and 4 compounds (+)-catechin, (**1**) (40 mg), (−)-epicatechin (**2**) (38 mg), (+)-gallocatechin (**3**) (72 mg) and (−)-epicatechin (**4**) (85 mg) were obtained and identified from their spectral data and reference to the literature [8].

(+)-Catechin (**1**). Off-white fine needles were obtained from H_2O, mp 177°. R_f (A) 0.47, R_f (B) 0.51. $[M+H]^+$: *m/z* 291.



R = H/OH

15 n = 2
17 n = 3 or 4
18 n = 4 or 5

^{1}H NMR (d_6-Me_2CO): δ6.89 (1H, J = 2.0 Hz, H-2'), 6.79 (1H, d, J = 8.1 Hz, H-5'), 6.75 (1H, dd, J = 2.0, 8.1 Hz, H-6'). 6.02 (1H, d, J = 2.3 Hz, H-8), 5.87 (1H, d, J = 2.3 Hz, H-6), 4.55 (1H, d, J = 6.7 Hz, H-2), 3.99 (1H, m, H-3), 2.91 (1H, dd, J = 5.5, 16.1 Hz, H-6), 2.53 (1H, dd, J = 8.4, 16.1 Hz, H-4'). ^{13}C NMR (d_6-Me_2CO): δ155.70 (C-8a), 157.17 (C-5), 156.86 (C-7), 145.69 (C-3'), 145.63 (C-4'), 132.20 (C-1'), 120.02 (C-6'), 115.68 (C-2'), 115.23 (C-5'), 100.65 (C-5a), 96.20 (C-8), 95.47 (C-6), 82.71 (C-2), 68.36 (C-3), 28.81 (C-4).

(−)-*Epicatechin* (**2**). Off-white fine needles were obtained from H_2O, mp 218° (decomp.) R_f (A) 0.37, R_f (B) 0.51. [M + H]$^+$: m/z 291. ^{1}H NMR (d_6-Me_2CO): δ7.05 (1H, d, J = 2.09 Hz, H-2'), 6.83 (1H, dd, J = 2.0, 8.1 Hz, H-6'), 6.78 (1H, d, J = 8.1 Hz, H-5'), 6.02 (1H, d, J = 2.3 Hz, H-8), 5.91 (1H, d, J = 2.3 Hz, H-6), 4.88 (1H, s, H-2), 4.20 (1H, m, H-3), 2.86 (1H, dd, J = 4.6, 16.6 Hz, H-4), 2.73 (1H, dd, J = 3.2, 16.6 Hz, H-4'). ^{13}C NMR (d_6-Me_2CO): δ157.56 (2C, C-5, 8a), 157.09 (C-7), 145.40 (C-3), 145.28 (C-4'), 132.23 (C-1'), 119.22 (C-6'), 115.47 (C-2'), 115.25 (C-5'), 99.79 (C-5a), 96.26 (C-8), 95.72 (C-6), 79.42 (C-2), 66.96 (C-3), 28.98 (C-4).

(+)-*Gallocatechin* (**3**). An off-white amorphous powder, R_f (A) 0.50, R_f (B) 0.55. [M + H]$^+$: m/z 307. ^{1}H NMR (d_6-Me_2CO): δ6.45 (2H, s, H-2', H-6'), 6.01 (1H, d, J = 2.3, H-8), 5.87 (1H, d, J = 2.3, H-6), 4.51 (1H, d, J = 7.3, H-2), 3.95 (1H, m, H-3), 2.86 (1H, dd, J = 5.4, 16.1 Hz, H-4), 2.52 (1H, dd, J = 8.0, 16.1 Hz, H-4'). ^{13}C NMR (d_6-Me_2CO): δ157.55 (C-8a), 157.08 (C-7), 156.72 (C-5), 146.14 (2C, C-3', C-5'), 133.19 (C-4'), 131.39 (C-1'), 107.20 (2C, C-2', C-6'), 100.47 (C-5a), 96.00 (C-8), 95.33 (C-6), 82.62 (C-2), 68.22 (C-3), 28.33 (C-4).

(−)-*Epigallocatechin* (**4**). An off-white amorphous powder, R_f (A) 0.45, R_f (B) 0.40. [M + H]$^+$: m/z 307. ^{1}H NMR (d_6-Me_2CO): δ6.57 (2H, s, H-2', H-6'), 6.01 (1H, d, J = 2.3 Hz, H-8'), 5.91 (1H, d, J = 2.3 Hz, H-6), 4.82 (1H, s, H-2), 4.19 (1H, m, H-3), 2.85 (1H, dd, J = 4.6, 16.5 Hz, H-4), 2.72 (1H, dd, J = 3.3, 16.5 Hz, H-4'). ^{13}C NMR (d_6-Me_2CO): δ157.52 (2C, C-7, C-8a), 157.09 (C-5), 146.14 (2C, C-3', 5'), 132.94 (C-4'), 131.56 (C-1'), 106.77 (2C, C-2', C-6'), 99.93 (C-5a), 96.25 (C-8), 95.76 (C-6), 79.46 (C-2), 67.03 (C-3), 28.82 (C-4).

Isolation and identification of dimers in fr. II. Fr. II was repurified on Sephadex LH-20 (1 × 50 cm) using $CHCl_3$–EtOH (4:1) to afford procyanidin B-1 (**5**) (46 mg) and procyanidin B-4 (**6**) (73 mg). Two more compounds catechin-(4α→8)-epigallocatechin (**7**) and gallocatechin-(4α→8)-epicatechin (**8**) were then obtained simultaneously. Prolonged elution with EtOH gave gallocatechin-(4α→6)-epigallocatechin (**9**) (360 mg). Further sepn of **7** (160 mg) and **8** (83 mg) was effected by reverse-phase HPLC (as described above). Compound **9** was also repurified by HPLC.

Procyanidin B-1 (**5**). An off-white amorphous solid. R_f (A) 0.51, R_f (B) 0.30. [M + H]$^+$: m/z 579. ^{1}H NMR (d_6-Me_2CO): δ6.60–7.20 (6H, m), 5.90–6.10 (3H, m). 5.05 (1H, s), 4.75 (2H, m), 4.10 (2H, m), 2.70–3.00 (2H, m).

Acid-catalysed thiolytic degradation of (**5**). A mixt. of **5** (30 mg), toluene-α-thiol (3 ml) and HOAc (3 ml) in EtOH (20 ml) was refluxed for 24 hr in N_2. The reaction mixt. was concd under red. pres. and the residue applied to a silica gel column (1 × 50 cm). The elution started with n-hexane to remove the excessive thiol. Further elution with $CHCl_3$–MeOH (from 10:0 to 7:3) gave epicatechin 4-benzylthioether (**10**) (11 mg) and catechin (**1**) (8 mg). The separation was monitored by TLC on silica gel. The TLC was performed in $CHCl_3$–MeOH (4:1) and visualized under UV_{254} and using 1% dodeca-molybdophosphoric acid in EtOH.

Epicatechin 4-benzylthioether (**10**). An oily off-white solid, R_f (A) 0.38, R_f (B) 0.86. ^{1}H NMR (d_6-Me_2CO): δ7.21–7.50 (5H, m), 7.05 (1H, d, J = 2.0 Hz), 6.82 (1H, d, J = 8.1 Hz), 6.79 (1H, dd, J = 2.0, 8.1 Hz), 6.04 (1H, d, J = 2.3 Hz), 5.90 (1H, d, J = 2.3 Hz), 5.30 (1H, s), 4.09 (1H, s), 4.03 (1H, d, J = 13.4 Hz), 4.02 (1H, d, J = 13.4 Hz), 3.93 (1H, m).

Procyanidin B-4 (**6**). A pale-brown amorphous powder. R_f (A) 0.50, R_f (B) 0.40 [M + H]$^+$: m/z 579. ^{1}H NMR (d_6-Me_2CO): δ6.55–7.20 (6H, m), 5.82–6.19 (3H, m), 4.89 and 5.02 (1H, s), 4.53 and 4.70 (1H, d, J = 7.7 Hz), 4.39 and 4.45 (1H, m and d, J = 8.9 Hz), 4.39 and 4.53 (1H, m), 4.11 and 4.26 (1H, $br\ s$), 2.71–2.98 (2H, m). The thiolytic degradation of **6** (40 mg) under the same conditions as described above furnished catechin 4-benzylthioether (**11**) (17 mg) and epicatechin (**2**) (12 mg).

Catechin 4-benzylthioether (**11**). An oily off-white solid. R_f (A) 0.43, R_f (B) 0.89. ^{1}H NMR (d_6-Me_2CO): δ7.19–7.41 (5H, m), 6.93 (1H, s), 6.78 (2H, s), 6.00 (1H, d, J = 2.3 Hz), 5.80 (1H, d, J = 2.3 Hz), 4.91 (1H, d, J = 9.7 Hz), 4.35 (1H, d, J = 3.4 Hz), 4.14 (1H, m), 4.21 (1H, d, J = 12.5 Hz), 4.04 (1H, d, J = 12.5 Hz).

Catechin-(4α→8)-epigallocatechin (**7**). A pale-brown amorphous powder, R_f (A) 0.42, R_f (B) 0.29. R_t (HPLC): 12.8 min. [M + H]$^+$: m/z 595. ^{1}H NMR (d_6-Me_2CO): δ6.37–7.03 (1H, m), 5.83–6.15 (3H, m), 4.91 and 4.99 (1H, s), 4.21–4.71 (4H, m), 2.72–2.99 (2H, m). ^{13}C NMR (d_6-Me_2CO): δ154.6–158.2 (6C), 145.3–145.9 (4C), 131.2–132.6 (2C), 128.2–128.5 (2C), 118.6–120.5 (2C), 114.5–115.8 (2C), 106.2–107.9 (3C), 95.9–97.1 (3C), 83.4, 79.6, 73.2, 66.8, 38.0, 29.8. Thiolytic degradation of (**7**) (40 mg) gave catechin 4-benzylthioether (**11**) (16 mg) and epigallocatechin (**4**) (11 mg).

Gallocatechin-(4α→8)-epicatechin (**8**). A pale-brown amorphous powder. R_f (A) 0.42, R_f (B) 0.29. R_t (HPLC): 15.2 min. [M + H]$^+$: m/z 595. ^{1}H NMR (d_6-Me_2CO): δ6.41–7.28 (5H, m), 5.85–6.25 (3H, m), 4.84 and 4.89 (1H, s), 4.29–4.74 (4H, m), 2.71–2.98 (2H, m). ^{13}C NMR (d_6-Me_2CO): 154.4–157.9 (6C), 145.1–145.6 (4C), 131.2–132.6 (3C), 119.0–119.9 (1C), 114.6–115.6 (2C), 106.3–107.8 (4C), 95.3–97.2 (4C), 83.8, 79.0, 73.0, 68.1, 38.3, 30.2. The thiolytic degradation of **8** (35 mg) under the same conditions as described

above produced gallocatechin 4-benzylthioether (**12**) (13 mg) and epicatechin (**2**) (9 mg).

Gallocatechin 4-benzylthioether (**12**). An oily off-white solid, R_f (A) 0.41, R_f (B) 0.73. ^{1}H NMR (d_6-Me_2CO): δ 7.19 7.44 (5H, *m*), 6.52 (2H, *s*), 6.01 (1H, *d*, J = 2.3 Hz), 5.81 (1H, *d*, J = 2.3 Hz), 4.89 (1H, *d*, J = 9.6 Hz), 4.36 (1H, *d*, J = 4.3 Hz), 4.13 (1H, *d*, J = 12.4 Hz).

Gallocatechin-(4α→6)-epigallocatechin (**9**). A pale-brown amorphous solid, R_f (A) 0.46, R_f (B) 0.25. R_t (HPLC): 11.6 min. $[M+H]^+$ and $[M+Na]^+$: *m/z* 611 and 633. ^{1}H NMR (d_6-Me_2CO): δ 6.70 (2H, *s*, H-2′, H-6′b), 6.58 (2H, *s*, H-2′, H-6′t), 6.02 (1H, *s*, H-8b), 5.86 (1H, *d*, J = 2.4 Hz, H-8t), 5.84 (1H, *d*, J = 2.4 Hz, H-6t), 4.91 (1H, *s*, H-2b), 4.66 (1H, *d*, J = 7.8 Hz, H-2t), 4.52 (1H, *m*, H-3t), 4.36 (1H, *d*, J = 9.6 Hz, H-4t), 4.24 (1H, *br s*, H-3b), 2.93 (1H, *dd*, J = 4.6, 16.8 Hz, H-4b), 2.80 (1H, *dd*, J = 2.7, 16.8 Hz, H-4′b). ^{13}C NMR (d_6-Me_2CO): δ 158.2, 157.2, 156.9, 155.2, 155.1, 154.5, 146.0 (2C), 145.9 (2C), 133.6, 132.2, 131.5, 131.2, 107.9 (3C), 106.2 (2C), 97.1 (3C), 95.9 (2C), 83.3, 79.6, 73.2, 66.7, 38.0, 30.1. The treatment of **9** (30 mg) with toluene-α-thiol under the same conditions as described above afforded gallocatechin 4-benzylthioether (**12**) (12 mg) and epigallocatechin (**4**) (8 mg).

Isolation and identification of trimers in fr. III. Repurification of fr. III was conducted on reverse-phase HPLC under the same conditions as described above, and two compounds (**13**) (90 mg) and (**14**) (130 mg) were obtained.

Catechin-(4α→8)-gallocatechin-(4α→6)-gallocatechin (**13**). A pale-brown amorphous powder, R_f (A) 0.31, R_f (B) 0.15. R_t (HPLC): 11.2 min. $[M+H]^+$ and $[M+Na]^+$: *m/z* 899 and 921. ^{1}H NMR (d_6-Me_2CO): δ 6.38–6.98 (7H, *m*), 5.79–6.28 (4H, *m*), 3.96–5.16 (8H, *m*), 2.72–3.02 (2H, *m*). ^{13}C NMR (d_6-Me_2CO): 154.9–157.9 (9C), 145.1–145.8 (6C), 128.6–133.3 (5C), 117.9–120.8 (1C), 114.9–116.2 (2C), 105.6–108.0 (8C), 95.3–99.6 (5C), 82.8–84.2 (3C), 71.5–74.2 (1C), 67.0 (2C), 39.2 (2C), 30.1 (1C). Treatment of **13** (45 mg) toluene-α-thiol furnished catechin 4-benzylthioether (**11**) (10 mg), gallocatechin 4-benzylthioether (**12**) (10 mg) and gallocatechin (**3**) (8 mg).

Gallocatechin-(4α→8)-gallocatechin-(4α→8)-epigallocatechin (**14**). A pale-brown amorphous powder, R_f (A) 0.31, R_f (B) 0.15. R_t (HPLC): 12.6 min. $[M+H]^+$: *m/z* 915. $[M+Na]^+$: *m/z* 937. ^{1}H NMR (d_6-Me_2CO): δ 6.51–6.69 (6H, *m*), 5.79–6.02 (4H, *m*), 3.96–4.91 (6H, *m*), 2.81–2.98 (2H, *m*). ^{13}C NMR (d_6-Me_2CO): δ 155.1–157.5 (9C), 145.2–145.7 (6C), 130.8–133.1 (6C), 105.9–108.7 (9C), 95.8–97.3 (6C), 82.9–84.0 (2C), 78.9–79.6 (1C), 73.5–74.1 (1C), 65.9 (2C), 38.5 (2C), 29.9 (1C). Thiolytic degradation of **14** (50 mg) gave gallocatechin 4-benzylthioether (**12**) (24 mg) and epigallocatechin (11 mg).

Identification of tetramers (**15**) *in fr. IV.* A pale-brown amorphous powder. R_f (A) 0.21, R_f (B) 0.10. R_t (HPLC): 6–8 min. FAB-MS $[M+H]^+$ and $[M+Na]^+$: *m/z* 1171, 1193; 1187, 1209; 1203, 1225; 1219, 1241. Treatment of (**15**) (60 mg) gave the thioethers (**10**–**12**) and epigallocatechin 4-benzylthioether (**16**) and the flavan-3-ols (**1**–**4**).

Epigallocatechin 4-benzylthioether (**16**). An oily off-white solid, R_f (A) 0.27, R_f (B) 0.68. ^{1}H NMR (d_6-Me_2CO): δ 7.19–7.46 (5H, *m*), 6.55 (2H, *s*), 6.02 (1H, *d*, J = 2.3 Hz), 5.90 (1H, *d*, J = 2.3 Hz), 5.21 (1H, *s*), 4.11 (1H, *s*), 4.04 (1H, *d*, J = 13.4 Hz), 4.01 (1H, J = 13.4 Hz), 4.00 (1H, *m*).

Determination of the composition and molecular weight of the oligomers. The tetrameric and oligomeric proanthocyanidins obtained from frs IV–VI were treated with toluene-α-thiol using the same method as described above except that the reaction was maintained for 48 hr. After removal of the solvent, the residue was applied to a silica gel column. The excessive thiol was removed with elution of *n*-hexane. A variety of the thioethers and flavan-3-ols were obtained using $CHCl_3$–MeOH (7:3). All collected frs were combined. After evapn of the solvents, the flavan-3-ols and their thioether derivatives were analysed using ^{1}H NMR spectroscopy in d_6-Me_2CO. Accurate integration of the characteristic H-2 signals was made from which the relative amounts of each component in the mixt. were determined. Determination of the ratio of the flavan-3-ols to their thioether derivatives led to an estimate of the average M_r of the proanthocyanidins.

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