Phytochemistry, Vol. 23, No. 8, pp. 1745–1752, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00
Pergamon Press Ltd.

OCCURRENCE OF PROCYANIDINS IN *NELIA MEYERI*

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(*Revised received* 28 *November* 1983)

Key Word Index—*Nelia meyeri*; Mesembryanthemaceae; 4-arylflavan-3-ol; procyanidins; ^{1}H NMR parameters; polymer sequence; degradative methods.

Abstract—A novel natural 4-arylflavan-3-ol has been obtained from leaves of *Nelia meyeri*. The same extract contains two procyanidin dimers, B-2 and B-5, and trimer C-1. ^{1}H NMR studies furnish diagnostic chemical shift parameters for determining the points of linkage in these compounds. The polymeric procyanidin isolated consists mainly of monomeric units with the 2,3-*cis* configuration and is considered to represent a pentamer or hexamer. The flavan-3-ol sequence of the polymer is proposed on the basis of partial degradation.

INTRODUCTION

Nelia meyeri Schwant. is a rare South African succulent which grows in Namaqualand, particularly at Komaggas and in the Richtersveld [1]. Recent investigations revealed the presence of condensed tannins [2] and evidence of the first 4-arylflavan-3-ol from a natural source [3]. The aim of the present study was to examine in more detail the procyanidins present in *Nelia meyeri* in terms of composition, stereochemical aspects and flavan-3-ol sequence.

RESULTS AND DISCUSSION

Continued investigation of phenolic components from leaves of *Nelia meyeri* has shown that with the exception of one polymer examined, all bi- and triflavanoids are exclusively based on flavan-3-ol units with 2,3-*cis* stereochemistry. (−)-Epicatechin (**1a**) and (+)-catechin (**2a**) were isolated and apparently represent the precursors of associated condensed tannins. The approximate ten-fold excess of **1a** correlates with the absence of (+)-catechin units in bi- and triflavanoids and its limited presence in oligomers.

Reinvestigation of the dimeric fraction afforded the first naturally occurring 4-arylflavan-3-ol (**11a**) [3] in a 4.3% yield of associated procyanidins **3a** and **4a**. The structure of **11a** was derived from the analysis of its heptamethyl ether monoacetate (**11b**) by mass spectrometry ($[M]^+$ 554) and ^{1}H NMR spectroscopy in $CDCl_3$ recorded at elevated temperature (100°) to ensure sharpening of all resonances. Spin-decouplings established the allocations of resonances and also demonstrated for the first time that secondary couplings of H-2 ($J_4 = 0.75$ Hz) may be attributed to long-range couplings with H-4 rather than to the influence of B-ring protons, as indicated by the decoupling of H-4 (δ 4.59 *d*) and irradiation of B-ring protons [3]. This observation is explicable in terms of an exception to the *W*-rule (coupling through σ-bonds) [4]. The relationship between H-2 and H-4 (C) is also evident in the ^{1}H NMR spectrum of dimer **3b** ($J_4 = 0.6$ Hz) recorded under the same solvent and temperature conditions as **11b**. Coupling constants reaffirmed the 2,3-*cis*-3,4-*trans* stereochemistry ($J_{2,3} = 1.8$ Hz, $J_{3,4} = 2.0$ Hz) [5–7] on the assumption of a half-chair conformation of the heterocyclic C-ring, while the absolute configuration at C-4 was supported by circular dichroism (cf. Fig. 1) [8–10].

The synthesis of **11b** was accomplished by condensation of the flavan-3,4-diol (**15**) prepared from tetramethyl ether (−)-epicatechin according to the procedure of Bett *et al.* [11] with phloroglucinol under acidic conditions, thus confirming its structure. Characterization of the 4-arylflavan-3-ol (**11b**), which may be regarded as a prototype, represents an extension of the known series of natural biflavanoids.

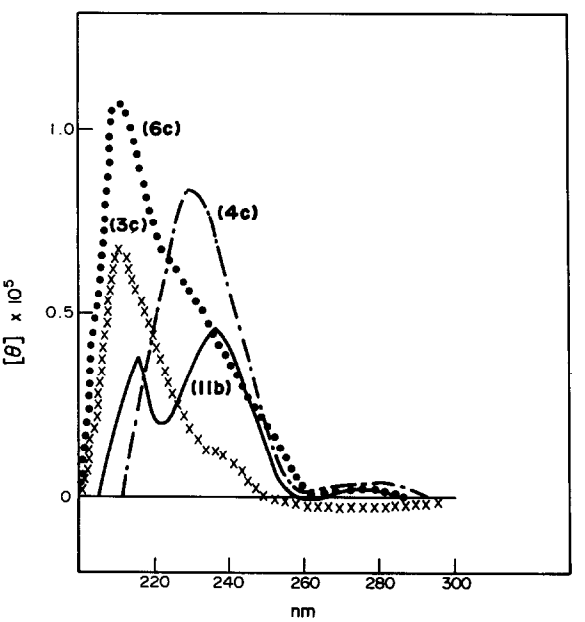
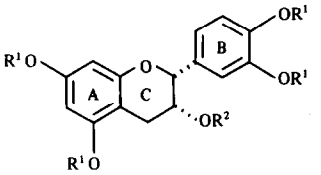
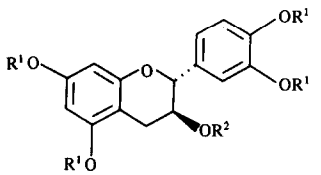


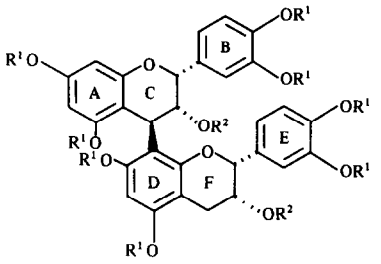
Fig. 1. CD spectra of procyanidin acetates **3c**, **4c** and **6c** and the methyl ether acetate **11b**.



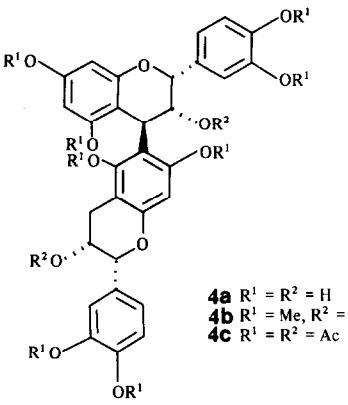
1a $R^1 = R^2 = H$
1b $R^1 = R^2 = Ac$



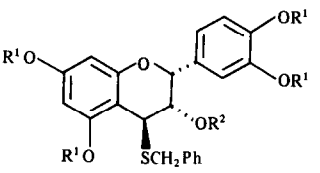
2a $R^1 = R^2 = H$
2b $R^1 = R^2 = Ac$



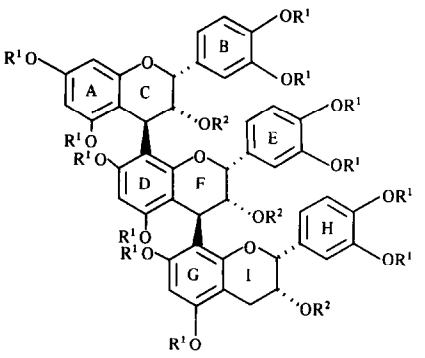
3a $R^1 = R^2 = H$
3b $R^1 = Me, R^2 = Ac$
3c $R^1 = R^2 = Ac$



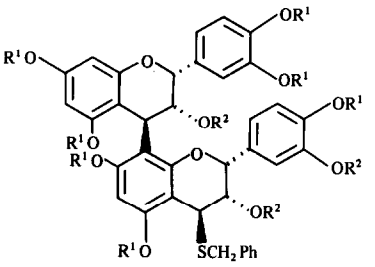
4a $R^1 = R^2 = H$
4b $R^1 = Me, R^2 = Ac$
4c $R^1 = R^2 = Ac$



5a $R^1 = R^2 = H$
5b $R^1 = R^2 = Ac$



6a $R^1 = R^2 = H$
6b $R^1 = Me, R^2 = Ac$
6c $R^1 = R^2 = Ac$



7a $R^1 = R^2 = H$
7b $R^1 = R^2 = Ac$

The separation of distinct procyanidins required acetylation as a convenient method to facilitate their isolation by preparative TLC after initial fractionation of the free phenols on Sephadex LH-20 [12]. Examination of biflavanoid fractions revealed the presence of procyanidins B-2 (**3a**) and B-5 (**4a**), their structures, and relative and absolute configurations established by mass spectrometry of derivatives **3b** and **4b**, respectively, NMR and CD measurements of **3c** and **4c** providing spectral characteristics consistent with reported data [10, 12–15]. In conjunction with NMR evidence, corroborative proof of structures **3a** and **4a** was provided by thiolysis of their free phenols [12], yielding the products **1a** and **5a** with spectral properties described previously [12].

The ^{1}H NMR spectra of **3c** and **4c** at ambient temperatures exhibited dynamic rotational isomerism [12, 16, 17] as shown clearly by line-broadening in the heterocyclic region and duplication of acetyl signals. However, temperature elevations (170°, DMSO-d_6), required in order to obtain sharp spectra, indicates, due to the persistence of line-broadening at this temperature, an insufficient activation energy for 'fast' rotation about interflavanoid bonds of derivatives with bulky substituents like acetyl groups.

While methyl ether acetates provide characteristic chemical shifts of H-6 and H-8 in $CDCl_3$ (100°) [18, 19], the corresponding resonances of the acetylated procyanidins are not amenable to similar analytical parameters previously established [20]. The ^{1}H NMR distinction between the [4,8]- and [4,6]-bonding positions in the spectra of peracetates is either based on rotamer populations [15] or on relative chemical shifts of the AB-quartet of ring A [21, 22]. Use of this parameter is consistent with the observed downfield shift ($\Delta\delta$ 0.5–0.6 ppm; **4c**) relative to the same system in the spectrum of the [4,8]-isomer (**3c**), possibly due to shielding effects in the latter instance caused by the aromatic E-ring, as concluded from molecular models. However, this parameter is restricted to dimeric procyanidin acetates with (−)-epicatechin as the 'upper' unit, since analysis of ^{1}H NMR spectra of B-3 acetate displays this AB-system strongly deshielded at δ 6.60–6.61 relative to its counterpart of B-6 acetate at δ 5.95 and 6.30 (unpublished results), which does not correlate with the above statement [22]. Accordingly, knowledge of the stereochemistry of the 'upper' flavan unit is required for this parameter. This factor necessitates additional diagnostic ^{1}H NMR parameters. Thus, chemical shift differences between the D-ring singlet, attributed to H-6 or H-8, and the forementioned AB-quartet are similarly significant and fall within the range of δ 0.03–0.17 for the [4,6]-linked procyanidins studied and δ 0.42–0.66 for their [4,8]-linked counterparts (cf. Table 1). Considerations on this basis, presently under investigation, may provide chemical shift differences of general diagnostic value. In an extended study (cf. Table 1), the significance of these ^{1}H-shift parameters was established irrespective of the type of solvent or temperature despite absolute shift differences under varying conditions. However, chemical shift differences are in no way enhanced in comparison with former conditions ($CDCl_3$, 30°).

The [4,8]-linked dimeric procyanidin (**3a**) predominates over its [4,6]-linked isomer (**4a**) in the plant extract by a factor of 10:1.

Compound (**6a**), co-eluted with B-5 (**4a**) from Sephadex LH-20 but also obtained from the following fractions, represents the [4,8:4,8]-tri-(−)-epicatechin (C_1). The structure of **6a** was evident from a combination of spectroscopic data and thiolytic degradation, as for procyanidins B-2 and B-5 [12, 22]. Analysis of the ^{1}H NMR spectrum of derivative **6c** in $CDCl_3$, which exhibited *meta*-coupled doublets (δ 6.25 and 5.94, J = 2.3 Hz, A-ring) in addition to two singlets [δ 6.69 (G) and 6.64 (D)], indicated successive [4,8]-interflavanoid linkages. The chemical shifts and coupling constants of the heterocyclic protons of **6c** and **3c** were similar, but differed from each other by additional resonances {δ 5.19 [*s*, H-2 (I)], 5.47 [*m*, H-3 (I)], 4.69 [*s*, H-4 (F)]}* in the former attributable to a third flavanoid unit with 2,3-*cis* stereochemistry (J < 1.0 Hz). Similarly, substantial line-broadening in the ^{1}H NMR spectrum of trimer **6c** (DMSO-d_6 at 170°), particularly in the heterocyclic region, correlated with observations for B-2 and B-5. The CD spectra of the acetates **3c**, **4c** and **6c** were similar in that they exhibited high amplitude positive Cotton effects indicative of 4*R*-configurations in each instance (cf. Fig. 1) [8–10].

Polymeric proanthocyanidins have recently received considerable attention through the application of degradative studies, chiroptical methods and ^{13}C NMR [23–29]. The polymer fraction, isolated concurrently

*Assignments of the resonances of **6c** were based on extensive low-power spin-decoupling experiments.

Table 1. Chemical shifts of A- and D-ring protons and their respective chemical shift differences under varying conditions

Conditions	3c	$\Delta\delta$ { H-6 (D), H-6 (A) or H-6 (D), H-8 (A) }	4c	$\Delta\delta$ { H-8 (D), H-6 (A) or H-8 (D), H-8 (A) }
$CDCl_3$, 30°*	6.65 *s*		6.59 *s*	
	6.23 *d*	0.42	6.72 *d*	0.13
	6.00 *d*	0.65	6.56 *d*	0.03
DMSO, 170°	6.76 *s*		6.56 *s*	
	6.50 *d*	0.26	6.75 *d*	0.19
	6.47 *d*	0.29	6.61 *d*	0.05
Nitrobenzene-d_5, 170°	6.92 *s*	0.32	6.78 *s*	
	6.60 *br*†	0.32	6.95	0.17
			6.77 *d*	0.01
Nitrobenzene-d_5,* 30°	6.98 *s*		6.77 *s*	
	6.48 *d*	0.50	6.94 *d*	0.17
	6.43 *d*	0.55	6.86 *d*	0.09

*Main conformer.

†The expected *dd* appeared as a broad resonance at this temperature.

from Sephadex LH-20 and defined as discrete areas in several solvent systems, gave a positive cyanidin reaction. The phenolic composition of the chromatographically homogeneous polymer was also demonstrated by its alkali fusion, which gave phloroglucinol and protocatechuic acid, and by thiolysis (72 hr) [12], resulting in a range of products which were successfully resolved on Sephadex LH-20. These compounds comprised (−)-epicatechin (**1a**) representing the polymer chain termination unit and the thioethers **5a** and **7a** as extension units. All these products were consistent with mass and NMR spectral data [12] and, in the case of **7a**, with results obtained by partial thiolysis [12]. This reaction is analogous to the treatment of **6a** with toluene-α-thiol.

Thioether **7a** when examined as derivative **7b** by ^{1}H NMR spectroscopy in $CDCl_3$ at ambient temperatures displayed two sets of clearly resolved resonances of heterocyclic and phloroglucinol protons (A and D) indicative of rotational isomers. The chemical shifts of the methylene protons of the $-SCH_2$ group were similar to those of **5b** (δ 3.83 *d* and 4.12 *d*, J = 14.0 Hz). The relative 3,4-*trans* stereochemistry was confirmed by appropriate coupling constants ($J_{3,4} = J_{3',4'} = 2.0$ Hz) [5–7], and the absolute configuration at C-4 was evident from the CD spectrum (cf. Fig. 2) [8–10].

Finally, the above reaction afforded on further elution a mixture of two products, which were generated in minor proportions and tentatively identified as trimeric constituted thioethers **8** and **9** on the grounds of complete degradation by toluene-α-thiol to give (−)-epicatechin (**1a**) and the 4-benzylthioethers of (−)-epicatechin (**5a**) and (+)-catechin (**10**), established by subsequent desulphurization. Chromatographic comparison of **5a** and **7a** with **8** and **9** on cellulose plates supported their trimeric constitution.

The foregoing series of degradation products provides indirect evidence of the flavan-3-ol sequence of the procyanidin polymer of *Nelia meyeri*: (−)-epicatechin (**1a**) represents the 'terminal' unit and is considered to consist of the following three chain extender units; the position of an incorporated (+)-catechin unit is concluded from the two degradation products **8** and **9** obtained by partial thiolysis. The average chain length is approximately five or six flavan-3-ol units, as measured on the basis of the ratio of (−)-epicatechin (**1a**) and thioethers **5a** and **10** (1:4–5) [30], indicating $\bar{M}_n \sim$ 1500–1800. All attempts to determine the remainder unit(s) failed, since the control of the acid-catalysed degradation with toluene-α-thiol is apparently difficult due to random fission of interflavan bonds after prolonged reaction time. The predominance of the monomer with the 2,3-*cis* configuration in the polymer is reflected in the estimated ratio of thioethers **5a** and **10**, 4–5:1–2, obtained by complete degradation.

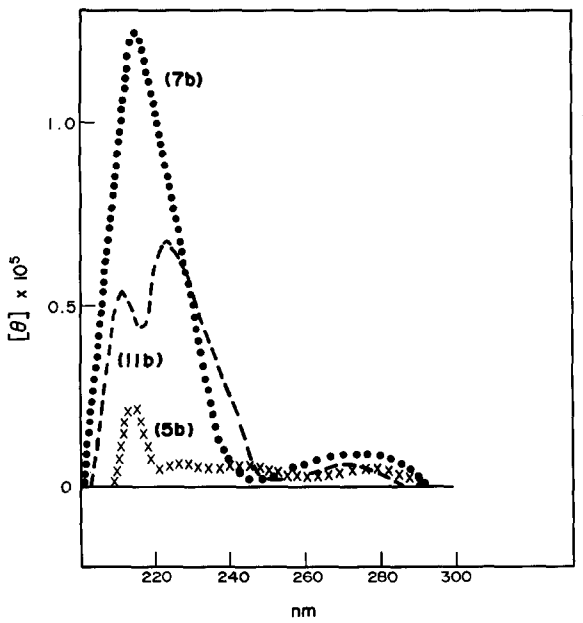


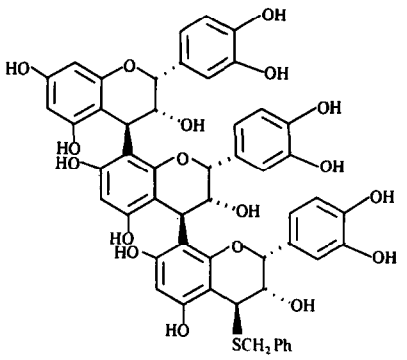
Fig. 2. CD spectra of the acetates **5b**, **7b** and **11c**.

In conclusion, the sequence of this polymer is based on the degradation pattern with respect to its purity entirely judged by chromatography. The remaining problems concerning the 3,4-stereochemistry may be rationalized to some extent on the assumption that all procyanidins consisting exclusively of (−)-epicatechin units possess, as far as we know, 3,4-*trans*-configurations, but this is limited by the inclusion of (+)-catechin.

The same reaction performed with phloroglucinol [15] generated the equivalent phloroglucinol analogues **11a** and **12a**, the latter tentatively identified by mass spectrometry of derivative **12b**, showing the molecular ion at *m/z* 940. Compound **11a** was identified by ^{1}H NMR and mass spectrometry, and confirmed by comparison of its optical rotation and circular dichroism with a synthetic reference sample. The aromatic protons H-3″ and H-5″ of the phloroglucinol moiety of **11b** appeared as an AB-quartet (δ 6.81 and 6.96, J = 2.5 Hz), indicating magnetic non-equivalence at ambient temperatures due to the chirality at C-4 coupled with restricted rotation.

In a further attempt to establish the chain sequence, the methylated polymer was treated with thioglycollic acid [31–33]. TLC analysis [33], after treatment with diazomethane, revealed the presence of two components at R_f 0.77 (**14b**) and 0.68 (**13b**) and products of higher complexity with low solubility (R_f 0–0.25). The oily products **13b** and **14b** were subsequently acetylated to give **13c** and **14c**, which failed to crystallize. Identification of the volatile methyl ether acetates **13c** and **14c** was based mainly on their mass spectra, which showed molecular ions *m/z* 492 ($[M]^+$, 0.3%, 0.2% respectively) in accordance with the proposed structures. The appearance of fragments *m/z* 432 ($[M-60]^+$, 1.3%) and *m/z* 327 ($[M-105]^+$, 0.5%), the former due to subsequent elimination of the thioglycollic residue, confirmed the suggested structures. Taken in conjunction with the chromatographic mobility, **13a** and **14a** are considered to represent flavan-4-yl-thioglycollates of (−)-epicatechin (**1a**) and (+)-catechin (**2a**), respectively, with undetermined stereochemistry at C-4. However, other degradation products of this reaction could not be characterized. Similar results were obtained with thioacetic acid [12].

In view of the results obtained from the degradation of the procyanidin polymer on treatment with either thioglycollic acid, phloroglucinol or toluene-α-thiol, the latter method is favoured due to the formation of products which can be readily separated and analysed, and hence the sequence of the original polymer may then be deduced. However, the conditions employed for the degradation



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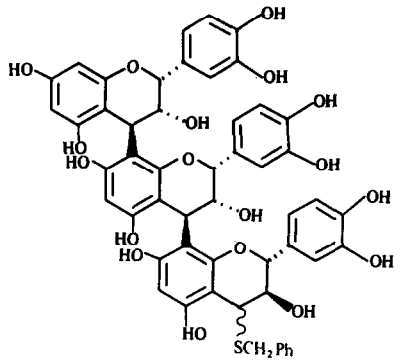

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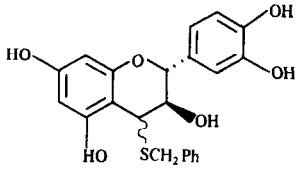

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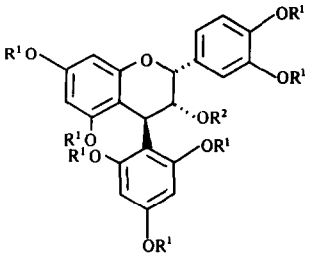
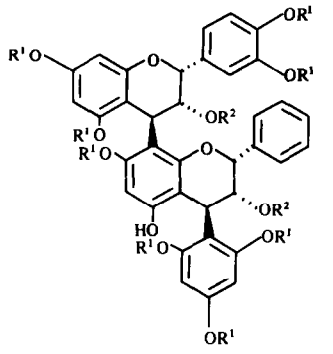
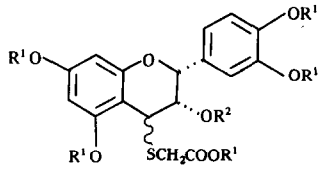


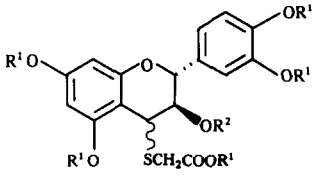
11a $R^1 = R^2$ H
11b R^1 = Me, R^2 = Ac
11c $R^1 = R^2$ = Ac



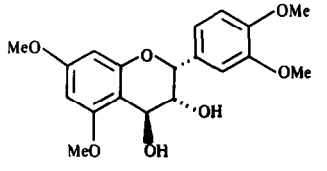
12a $R^1 = R^2$ = H
12b R^1 = Me, R^2 = Ac



13a $R^1 = R^2$ = H
13b R^1 = Me, R^2 = H
13c R^1 = Me, R^2 = Ac



14a $R^1 = R^2$ = H
14b R^1 = Me, R^2 = H
14c R^1 = Me, R^2 = Ac



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are not ideal, since the reaction does not proceed in a 'step-by-step cleavage' which permits unambiguous sequential determination of polymers. The problems associated with the acid-catalysed degradation in the presence of phloroglucinol are identical, and therefore this provides an alternative method while the degradative procedure with thioglycollic acid is unsuitable due to the fact that only the thioglycollates of the monomeric flavan-3-ols are amenable to analysis.

EXPERIMENTAL

NMR spectra were recorded in $CDCl_3$ with TMS as internal standard (unless stated otherwise). Mass spectra were obtained

with a Varian MAT 44S-Spectro System MAT 188 instrument. C and H analyses were performed by the Department of Organic Chemistry, Westfälische-Wilhelms-Universität, Münster. Mps are uncorr. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter, and circular dichroism measurements on a Dichrograph Mark III spectropolarimeter in MeOH. TLC was performed on DC-Plastikfolin Kieselgel 60 F_{254} (solvent system K: EtOAc–HCOOH–H_2O, 18:1:1) and on cellulose plates using (A) M HOAc and (B) *n*-BuOH–HOAc–H_2O, 14:1:5; chromatograms were sprayed with vanillin–HCl. Prep. plates (PLC) [Kieselgel PF_{254} (0.5 mm)] were air-dried and used without prior activation. Methylations were performed with an excess of diazomethane, while acetylations were carried out with Ac_2O–pyridine.

Isolation of procyanidins. Plant material (1.5 kg) was extracted with MeOH (× 4) and the combined extracts (4 l.) were evapd *in vacuo* to dryness. The residue was dissolved in H_2O (1 l.), washed with petrol (4 × 250 ml), and extracted with EtOAc (10 × 500 ml). The EtOAc extract was dried (Na_2SO_4) and evapd, yielding a brown solid (16 g). The crude water-soluble tannin was freeze-dried to obtain a red-brown powder (22 g). The EtOAc-soluble fraction was chromatographed in 2 g portions on Sephadex LH-20 (2.5 × 60 cm) using EtOH and finally MeOH as eluants. Fractions (15 ml) were collected and grouped as follows.

Monomeric flavan-3-ols. Fractions 59–73 represented a mixture [(−)-epicatechin (**1a**) and (+)-catechin (**2a**), R_f (K) 0.87] which gave two bands at R_f 0.52 and 0.39 on cellulose plates (0.4 mm) in H_2O–dioxane (20:2 g/g). The R_f 0.52 band was identical to (+)-catechin while the R_f 0.32 fraction gave (−)-epicatechin, identified by mp, mass and ^{1}H NMR spectra.

Dimeric procyanidins. Procyanidin B-2 (**3a**): From fractions 88–104 procyanidin B-2 (**3a**) was obtained as a brown amorphous powder (185 mg), R_f (K) 0.64, after rechromatography on Sephadex LH-20 in Me_2CO followed by precipitation from EtOAc with petrol. Acetylation and PLC separation (C_6H_6–Me_2CO, 4:1; R_f 0.46) gave the decaacetate (**3c**). (Found: C, 60.1; H, 4.7. Calc. for $C_{50}H_{46}O_{22}$: C, 60.1; H, 4.6%); $[\alpha]_{578}^{20}$ + 53.3° and $[\alpha]_{Na}^{20}$ + 51.2° (*c* 2.0; Me_2CO), lit. [13] $[\alpha]_{578}$ + 47°; ^{1}H and ^{13}C NMR were consistent with reported spectral data [12–13, 15]. CD, see Fig. 1. Methylation followed by acetylation yielded the octamethyl ether diacetate (3b), purified by chromatography on a silica gel column ($CHCl_3$–EtOAc, 9:1), showing $[M]^+$ 774 (28%).

Procyanidin B-5 (**4a**): The contents of tubes 127–138 showed the presence of procyanidin B-5 (**4a**), R_f (K) 0.78, and of the trimer (**6a**), R_f (K) 0.50. Chromatography on Sephadex LH-20 using Me_2CO as eluant afforded pure B-5 (29 mg). (Found: C, 62.1; H, 4.7. Calc. for $C_{30}H_{26}O_{12}$: C, 62.3; H, 4.6%.) Acetylation and PLC (C_6H_6–Me_2CO, 4:1; R_f 0.48) yielded the decaacetate (**4c**) as an amorphous solid. (Found: C, 59.6; H, 4.6. Calc. for $C_{50}H_{46}O_{22}$: C, 60.1; H, 4.6%); $[\alpha]_{578}^{20}$ + 80.2° (*c* 1.4; Me_2CO), lit. [34] $[\alpha]_{578}$ + 79.5°. ^{1}H NMR ($CDCl_3$): δ 1.84–2.31 (30 H, *m*, 10 × OAc), 2.92–2.96 [*m*, 2 × H-4 (F)], 4.16 and 4.34* [2 × *br s*, H-4 (C)], 5.15–5.43 [*m*, 4 × H, H-2 (C), H-3 (C), H-2 (F), H-3 (F)], 6.59 [*d*, *J* = 2.5 Hz, H-6 (A)], 6.60 [*s*, H-8 (D)], 6.72 [*d*, *J* = 2.5 Hz, H-8 (A)]†, 7.15–7.31 [6 H, *m* (B and E)]. ^{13}C NMR data were similar to those reported previously [15]. CD, see Fig. 1. The octamethyl ether diacetate (**4b**) was obtained by methylation of **4a**, followed by acetylation and purification by PLC (C_6H_6–Me_2CO, 4:1; R_f 0.61), showing $[M]^+$ 774 (21%).

Trimers. Procyanidin C-1: Fractions 139–161 afforded procyanidin C 1 (**6a**), R_f (K) 0.50, as a light-brown solid (142 mg). (Found: C, 62.3; H, 4.7. Calc. for $C_{45}H_{38}O_{18}$: C, 62.3; H, 4.4%.) Acetylation and PLC (C_6H_6–Me_2CO 4:1; R_f 0.29) yielded the peracetate **6c** as a white amorphous powder from hexane. (Found: C, 60.1; H, 4.7. Calc. for $C_{75}H_{68}O_{33}$: C, 60.1; H, 4.6%.) ^{1}H NMR ($CDCl_3$): δ 1.37–2.37 (*m*, 15 × OAc), 3.00 [*m*, 2 × H-4 (I)], 4.66 [*s*, H-4 (C)], 4.69 [*s*, H-4 (F)], 4.76 [*s*, H-2 (F)], 5.11 [*m*, H-3 (C)], 5.19 [*s*, H-2 (I)], 5.37 [*m*, H-2 (C)], 5.41 [*m*, H-3 (F)], 5.47 [*m*, H-3 (I)]‡, 5.94 [*d*, *J* = 2.2 Hz, H-6 (A)], 6.25 [*d*, *J* = 2.2 Hz, H-8 (A)], 6.64 and 6.69 [2 × *s*, 2 × H-6 (D and G)], 7.15–7.34 [9 H, *m* (B, E and H)]. $[M]^+$ 1160 (8%); CD, see Fig. 1.

(2R,3R,4S)-4-(2,4,6-*Trihydroxyphenyl*)*flavan*-3,5,7,3′4′-*pentaol* (**11a**). Methylation of the biflavanoid fraction (520 mg) followed by PLC separation (C_6H_6–Me_2CO, 4:1) gave three bands at R_f 0.56, 0.43 and 0.36. Acetylation of the R_f 0.56 band (48 mg) afforded the heptamethyl ether monoacetate (C_6H_6–Me_2CO, 9:1; R_f 0.57) (**11b**) as a solid (24 mg) from hexane. ^{1}H NMR and MS spectral data [3], and ^{13}C NMR [21] were consistent with reported data. CD, see (Fig. 1).

Synthesis of (2R,3R,4S)-3-*acetoxy*-4-(2,4,6-*trimethoxyphenyl*)-5,7,3′,4′-*tetramethoxyflavan* (**11b**). (*i*) *Synthesis of* 5,7,3′,4′-*tetramethoxyflavan*-3,4-*diol* (**15**): 5,7,3′,4′-Tetramethyl ether-(−)-epicatechin (0.7 g) was dissolved in dry C_6H_6 (12 ml), and $Pb(OAc)_4$ (1.5 g) was added. The reaction mixture was stirred at room temp. for 10 days and worked-up as described in ref. [11]. The solid (0.68 g) obtained on concn was applied to a neutral Al_2O_3 column. Elution with Et_2O (200 ml) gave tetramethyl ether-(−)-epicatechin (228 mg), and further elution with Et_2O afforded **15** (132 mg). $[\alpha]_{589}^{20}$ − 32.2° (*c* 1.0; $CHCl_3$), lit. [11] $[\alpha]_{589}$ − 33.7°; ^{1}H NMR ($CDCl_3$): δ 1.81 (*br s*, 1 × H, 3-OH), 2.95 (*br s*, 1 × H, 4-OH), 3.76, 3.82, 3.87, 3.89 (all *s*, 4 × OMe), 4.00 [*m*, H-3 (C)], 4.84 [*d*, *J* = 2.5 Hz, H-4 (C)], 5.09 [*s*, H-2 (C)], 6.12 [*d*, *J* = 2.5 Hz, H-6 (A)], 6.20 [*d*, *J* = 2.5, H-8 (A)], 6.84–7.12 [3H, *m*(B)]; MS *m/z* (rel. int.): 362 $[M]^+$ (13), 344 $[M - H_2O]^+$ (35), 316 (48), 301 (26), 180 (100), 165 (54), 151 (57), 137 (33).

(*ii*) *Condensation of* 5,7,3′,4′-*tetramethoxyflavan*-3,4-*diol* (**15**) *with phloroglucinol*: Flavan-3,4-diol (**15**) (100 mg) and phloroglucinol (200 mg) were dissolved in a mixture of 70% EtOH (20 ml) and HOAc (5 ml). The reaction mixture was heated briefly (50°, 5 min) and diluted with H_2O (100 ml). The soln was kept at 4° (48 hr) to give a ppt. (37 mg), which was methylated and acetylated to give **11b**, which had spectral properties identical to the natural product.

Degradation of procyanidins with toluene-α-thiol. Procyanidins **3a**, **4a** and **6a** (50–100 mg) were dissolved in EtOH (2 ml) and toluene-α-thiol (1 ml), and HOAc (0.5 ml) was added. The mixture was refluxed in N_2 for 24 hr, while the reaction was followed by 2D chromatography on cellulose plates, using systems (A) and (B). Evapn of the solvents and subsequent chromatography of the oily residue on Sephadex LH-20 ($CHCl_3$–PrOH 4:1; 10 ml fractions) afforded the flavan-3-ol (**1a**) (50–60% yields) from fractions 72–85, which was identified by co-chromatography with an authentic sample.

(2R,3S,4S)-4-*Benzylthioflavan*-3,5,7,3′,4′-*pentaol* (**5a**). The thioether **5a**, (60–65% yields) was obtained from fractions 103–121; $[\alpha]_{578}^{20}$ − 21° (*c* 1.2; EtOH), lit. [12] $[\alpha]_{578}$ − 21.1°. Acetylation and prep. TLC (C_6H_6–Me_2CO, 4:1; R_f 0.63) gave its pentaacetate (**5b**), mp 122°. (Found: C, 61.5; H, 5.0; S, 5.3. Calc. for $C_{32}H_{30}O_{11}S$: C, 61.7; H, 4.9; S, 5.1%); $[\alpha]_{578}^{20}$ − 37° (*c* 0.7; $CHCl_3$) lit. [12] $[\alpha]_{578}$ − 35.3°. ^{1}H NMR ($CDCl_3$): δ 1.83, 1.93, 2.25 and 2.30 (12H, 4 × *s*, OAc), 3.87 (*d*, *J* = 12.0 Hz, –S–CH_2–), 4.07 (*d*, *J* = 12.0 Hz, –S–CH_2–), 4.12 [*d*, *J* = 1.0 Hz, H-4 (C)], 5.26 [*d*, *J* = 1.0 Hz, H-3 (C)], 5.69 [*s*, H-2 (C)], 6.54 [*d*, *J* = 2.0 Hz, H-6

*Attributable to rotameric forms.

†The minor conformer was characterized by signals at δ 6.47 (*d*, *J* = 2.0 Hz), 6.61 (*s*) and 6.79 (*d*, *J* = 2.0 Hz).

‡Assignments to the C- and F-ring systems may be interchanged.

(A)], 6.65 [*d*, *J* = 2.0 Hz, H-8 (A)], 7.1–7.4 [8H, *m* (B and D)]. MS *m/z* (rel. int.): 622 $[M]^+$ (6), 5.62 (8), 498 $[M-124]^+$ (27), 471 (50), 456 (26), 439 (100), 428 (38), 397 (94), 373 (62), 355 (80), 314 (68), 303 (60), 288 (57), 271 (50), 223 (63), 181 (51), 165 (40); CD, see Fig. 2.

However, analogous treatment of the trimer **6a** with toluene-α-thiol revealed two additional compounds. Fractions 128–145 contained procyanidin B-2 (**3a**), R_f at 0.59 and 0.41 in H_2O–dioxane (10:1 g/g) and BAW (upper phase, 4:1:1) respectively, and fractions 150–162 afforded a compound [its peracetate showed $[\alpha]_{570}^{20}$ + 26.3° (*c* 0.2; $CHCl_3$)] presumed to be the dimeric thioether **7a** on the basis of prolonged reaction with toluene-α-thiol, yielding **1a** and **5a** as the only products, identified by analysis as described above.

Desulphurization of thioethers was achieved with Raney nickel (2 hr) to give (−)-epicatechin (**1a**).

Reactions of polymeric procyanidins. (i) Reactions of polymeric procyanidins with toluene-α-thiol. Polymeric procyanidin (3.0 g) obtained from Sephadex LH-20 chromatography was suspended in EtOH (20 ml) and toluene-α-thiol (6 ml), and HOAc (3 ml) was added. The mixture was refluxed in N_2 for 72 hr. The oil obtained on removal of solvents was subjected to CC as before. Fractions (20 ml) were collected and examined on cellulose plates in systems (A) and (B). By using these chromatograms as a reference, the test-tubes were grouped as follows:

Fractions	Components [R_f values in systems (A) and (B), respectively]
11–16	**1a** (0.35; 0.54)
17–46	**1a** and **5a**
47–67	**5a** (0.34; 0.78)
68–74	**5a** and **7a**
75–94	**7a** (0.56; 0.72)
120–150	**8** (0.38; 0.56) and **9** (0.34; 0.54)

While all the mixed fractions could be purified by rechromatography, sufficient separation of fractions 120–150 could not be achieved by multiple rechromatography. This mixture when completely degraded with toluene-α-thiol (7 days) gave two thioethers, one of which was shown to be **5a**, the second tentatively identified as benzylthiocatechin (**10**) on the basis of chromatographic behaviour and desulphurization affording (+)-catechin.

(2R,3S,4S)-4-*Benzylthioflavan*-3,5,7,3′,4′-*pentaol* (**5a**): Acetylation gave **5b**, identical to the corresponding product derived from degradation of **3a**. (2R,3R,4R)-2,3-Cis-3,4-trans-3,5,7,3′,4′-*pentahydroxy*-4-[(2R,3S,4S)-2,3-cis-3,4-trans-3,5,7,3′,4′-*pentahydroxy*-4-*benzylthioflavan*-8-*yl*]*flavan* (**7a**): The thioether **7a** (83 mg) was obtained from fractions 68–74. (Found: C, 63.4; H, 4.9; S, 4.4. Calc. for $C_{37}H_{32}O_{12}S$: C, 63.4; H, 4.6; S, 4.6%); $[\alpha]_{578}^{20}$ + 66.7° (*c* 0.5; EtOH), lit. [30] $[\alpha]_{578}$ + 64.1°. Acetylation gave the decaacetate **7b** as a solid from hexane after purification by prep. TLC (C_6H_6–Me_2CO, 4:1; R_f 0.45) (Found: C, 61.1; H, 4.8; S, 2.6. Calc. for $C_{57}H_{52}O_{22}S$: C, 61.1; H, 4.7; S, 2.9%.) ^{1}H NMR ($CDCl_3$): δ 1.72–2.31 (30 H, *m*, 10 × OAc), 3.83 (*d*, *J* = 14 Hz, –SCH_2–), 4.12 (*d*, *J* = 14 Hz, –SCH_2–), 4.15 [*d*, *J* = 2.0 Hz, H-4 (F)], 4.50 [*d*, *J* = 2.0 Hz, H-4 (C)], 4.66 [*s*, H-2 (F)], 5.09 [*m*, H-3 (F)], 5.34 [*m*, H-3 (C)], 5.53 [*s*, H-2 (C)], 6.06 [*d*, *J* = 2.3 Hz, H-6 (A)], 6.31 [*d*, *J* = 2.3 Hz, H-8 (A)], 6.97–7.41 [11H, *m*, (B, E and G)]. MS *m/z* (rel. int.): 1060 $[M-60]^+$ (0.7), 1001 $[M-2 \times HOAc]^+$ (0.7), 996 $[M-124]^+$ (1.2), 937 (15.2), 876 (9.2), 327 (1.8), 313 (11.6), 161 (41.8), 152 (18.6), 137 (7.3), 124 (53.9), 123 (44.9), 91 (100). CD, see Fig. 2.

(ii) Reaction of polymeric procyanidins with phloroglucinol: Polymeric procyanidin (5.0 g) and phloroglucinol (7.5 g) were dissolved in dioxan and 0.5 M HCl (150 ml; 1:1) and stirred in N_2 for 4 days. The soln was extracted with EtOAc (5 × 100 ml), dried, and evapd to afford an oil (8.0 g) which was chromatographed in portions (2.0 g) on Sephadex LH-20 (2 × 50 cm) with EtOH. Fractions (20 ml) 26–44 contained phloroglucinol and from fractions 45–55 (−)-epicatechin was obtained.

(2R,3R,4S)-4-(2,4,6-*Trihydroxyphenyl*)*flavan*-3,5,7,3′,4′-*pentaol* (**11a**): Fractions 62–87 contained compound **11a** as a light tan solid (212 mg). Acetylation and PLC (C_6H_6–Me_2CO, 4:1; R_f 0.54) yielded the octaacetate **11c** as a solid from hexane, mp 145–148°. (Found: C, 59.2; H, 5.6. Calc. for $C_{37}H_{34}O_{17}$: C, 59.2; H, 5.4%); $[\alpha]_{578}^{20}$ + 86.4° (*c* 0.2; $CHCl_3$), lit. [15] $[\alpha]_{578}$ + 93.6°. ^{1}H NMR ($CDCl_3$): δ 1.83, 1.87, 2.00, 2.25, 2.31 (24H, all *s*, 8 × OAc), 4.43 [*d*, *J* = 2.4 Hz, H-4 (C)], 5.12 [*m*, H-3 (C)], 5.44 [*s*, H-2 (C)], 6.59 [*d*, *J* = 2.5 Hz, H-6 (A)], 6.74 [*d*, *J* = 2.5 Hz, H-8 (A)], 6.81 [1H, *d*, *J* = 2.5 Hz (D)], 6.96 [1H, *d*, *J* = 2.5 Hz (D)], 7.15–7.31 [3H, *m* (B)]; $[M]^+$ 750 (3%); CD, see Fig. 2.

(iii) Reaction of polymeric procyanidins with thioglycollic acid: A mixture of methylated polymeric fraction (400 mg), H_2O (6 ml), and thioglycollic acid (8 ml) was heated (150°) for 4 hr in N_2. H_2O (15 ml) was added and the mixture was extracted with EtOAc (3 × 20 ml). The EtOAc extract was treated with $NaHCO_3$ soln (10%, 20 ml) and the pH adjusted to 9.0 with solid $NaHCO_3$. The aq. phase was acidified with HCl and extracted with Et_2O (5 × 50 ml) to give an oil (8 ml), which was dissolved in Me_2CO (40 ml) and methylated with Me_2SO_4 (2.5 ml) and dry K_2CO_3 (6 g). The oily product (3 ml) was resolved by multiple PLC (C_6H_6–Me_2CO, 9:1) giving two fractions at R_f 0.78 and 0.66.

2,3-Cis- *and* 2,3-trans-(3-*acetoxy*-5,7,3′,4′-*tetramethoxyflavan*-4-*yl-thio*)*acetate* (**13c**) *and* (**14c**). The R_f 0.78 fraction (5 mg) was acetylated and purified (C_6H_6–Me_2CO, 4:1) to give 7 mg of **14c**. Acetylation of the R_f 0.66 fraction (18 mg) yielded 22 mg of **13c**. The oily products were taken up in Et_2O and subjected to MS, showing *m/z* (rel. int.): 492 $[M]^+$ (0.3, 0.2%), 432 $[M-60]^+$, (1.3, 1.3), 369 (1.8, 1.4), 355 (2.5, 2.4), 327 (0.5, 0.5), 295 (4.7, 4.7), 267 (1.3, 1.4), 222 (3.1, 2.9), 167 (14.3, 14.8), 45 (100).*

Number average molecular weight determination of polymeric procyanidin [30]. Polymeric procyanidin (1.0 g) in EtOH (20 ml) containing toulene-α-thiol (3.0 ml) and HOAc (1.5 ml) was refluxed in N_2 for 7 days. The soln was worked up and chromatographed as described above to give (−)-epicatechin (118 mg) and **5a** and **10** (720 mg), indicating $\overline{M}_n$ 1500–1800.

Acknowledgements—The plant material of *Nelia meyeri* was kindly collected by Dr. H. Hartmann, Botanisches Institut, Hamburg. Helpful discussions with Prof. D. G. Roux and spin-tickling experiments by Prof. D. Ferreira are gratefully acknowledged.

*The base peak was due to the fragment $C_2H_5O^+$ assumed to be derived from ether.

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