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POLYMERIC PROANTHOCYANIDINS OF *PHOTINIA GLABRESCENS*, MODIFICATION OF MOLECULAR WEIGHT AND NATURE OF PRODUCTS FROM HYDROGENOLYSIS

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Key Word Index—*Photinia glabrescens*; Rosaceae; proanthocyanidin polymers; hydrogenolysis; interflavanoid bonds; structures; molecular size.

Abstract—The hydrogenolysis of *Photinia glabrescens* proanthocyanidin polymer was investigated. Among the low MW products identified were phloroglucinol, catechin, epicatechin, 1 - (3, 4 - dihydroxyphenyl) - 3 - (2, 4, 6 - trihydroxyphenyl) propan - 2 - ol and the procyanidin dimers B1 and B2. Initial reaction products may be related to the known structure of the polymer. The yield of the ethyl acetate-soluble fraction reached a maximum after about 3 hr of hydrogenation and the major oligomeric fractions had significantly lower MWs than the parent polymer.

INTRODUCTION

The escalating cost of oil in recent years has caused concern over the future supply and cost of phenols to the forest products industry and has brought about a renewed interest in the utilization of bark polyphenols for the manufacture of wood adhesives[1].

The major constituent of pine bark is condensed tannin or polymeric proanthocyanidin. These polymers have been shown to consist exclusively of flavan - 3 - ol units linked together by C–C bonds via C-4 of the pyran ring of a flavan unit to C-6 or C-8 of the phloroglucinol A ring of the adjacent unit[2, 3]. Problems caused by the high viscosity of these large polymers were encountered when polyflavanoid extracts were used in formulating wood adhesives[4]. Also the relatively inferior bond quality produced from tannin–formaldehyde adhesives, as compared with phenol–formaldehyde resins, has been suggested to be due to the size of the polymers inhibiting adequate cross-linking between polymer units[5]. Therefore, it appears that the size of these polymers will have to be reduced before they can be effectively used for adhesives. A selection of chemical methods[6–10] has been employed to cleave the interflavanoid bonds, and one of particular interest is concerned with the catalytic hydrogenation of procyanidin dimers[12]. Although catalytic hydrogenation of Douglas-fir bark fires has been described previously [13], a great deal remains to be understood as to what happens to the proanthocyanidin components when subjected to this reaction. The present study is involved with the hydrogenolysis of the polymeric proanthocyanidins (**3**) from *Photinia glabrescens* which have been shown in an earlier study[14] to consist exclusively of procyanidin units with the epicatechin (**1**) type stereochemistry and having catechin (**2**) as the terminal unit.

RESULTS AND DISCUSSION

The polymeric procyanidin was not cleaved under the mild hydrogenation conditions employed for the procyanidin dimers[12] even when the reaction conditions were maintained for over 4 days. However, interflavanoid bond cleavage occurred readily at elevated temperatures and pressures. The yields of lower MW products obtained by extraction of the reaction mixtures with ethyl acetate is shown in Fig. 1. At 1000 psi of hydrogen and 180° with palladium as catalyst the maximum yield of *ca* 75% of conversion to ethyl acetate solubles was achieved in 3 hr. Beyond this point the yield of the lower MW components declined with a corresponding increase in the appearance of an insoluble glassy material which no longer produced anthocyanidins on treatment with mineral acid. The progress of hydrogenolysis was monitored by sampling at regular intervals. The nature of the vanillin–hydrochloric acid reactive products and their relative yields (based on colour intensity of spots visualized with vanillin–hydrochloric acid spray) were determined by 2D-TLC on cellulose. The dimers B1 (**4**) and B2 (**5**) together with catechin, epicatechin, 1 - (3, 4 - dihydroxyphenyl) - 3 - (2, 4, 6 - trihydroxyphenyl)propan - 2 - ol (**6**) and phloroglucinol were identified by co-chromatography on 2D-TLC and HPLC on a reverse phase column with authentic samples. Authentic 1 - (3, 4 - dihydroxyphenyl) - 3 - (2, 4, 6 - trihydroxylphenyl)propan - 2 - ol was prepared by hydrogenolysis of epicatechin at 180° and at 1000 psi of hydrogen. Like the procy-

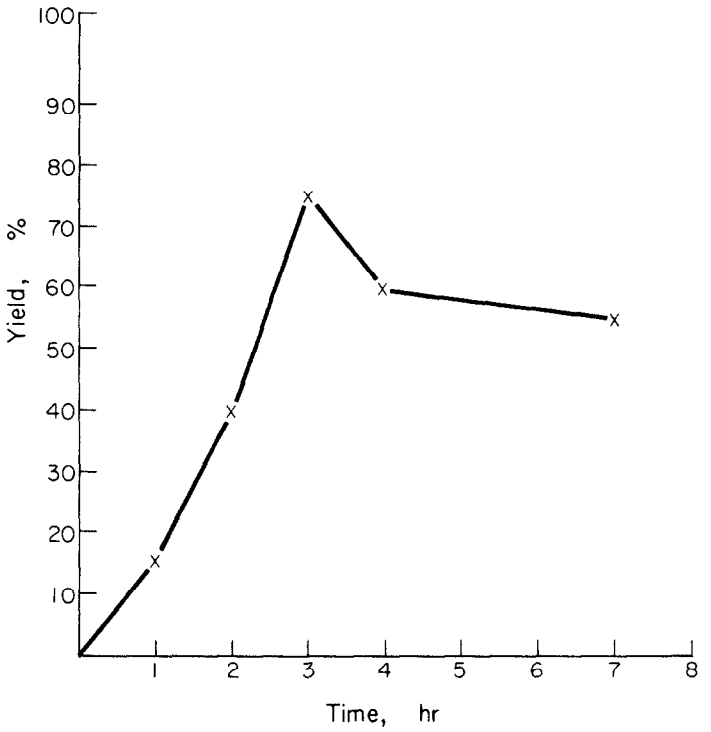
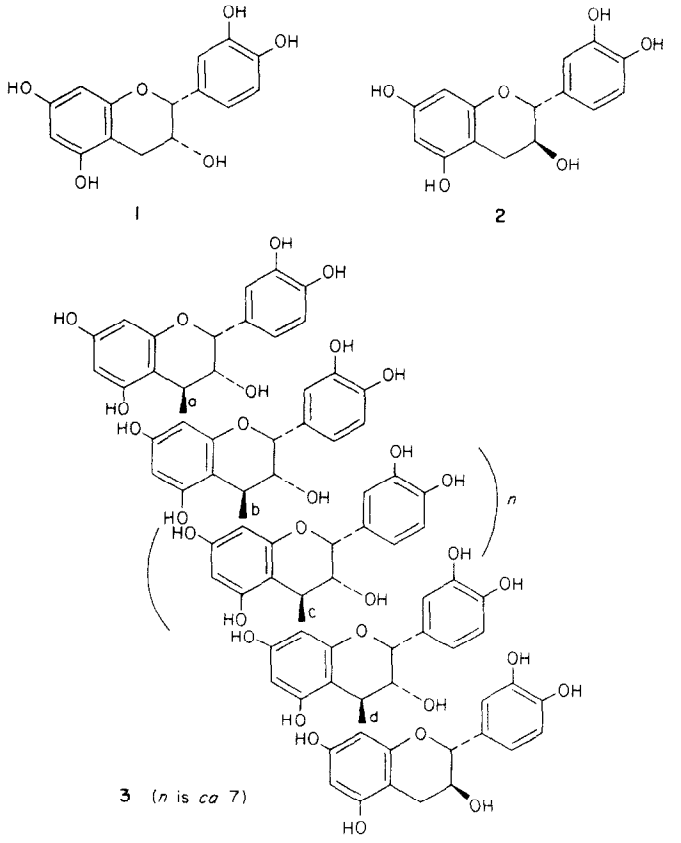
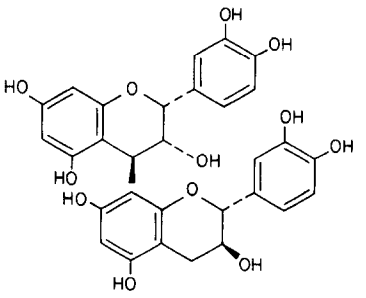


Fig. 1. Yield of ethyl acetate-soluble fraction vs time of hydrogenolysis at 1000 psi over palladium on carbon at 1000 psi and 180°.





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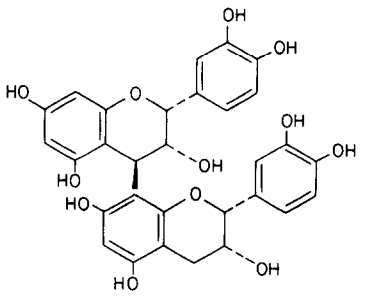

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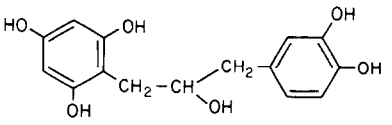

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anidin polymers, and contrary to an earlier report[12] the epicatechin did not react at room temperature and pressure, and even under the conditions presently employed only partial reaction took place to give the ring-opened compound (**6**) and some catechin, presumably via epimerization at C-2. **6** was characterized by comparison of its pentamethyl derivative with the 1 - (3, 4 - dimethoxyphenyl) - 3 - (2, 4, 6 - trimethoxyphenyl)propan - 2 - ol obtained from an independent synthesis via sodium–liquid ammonia reduction of tetramethyl epicatechin followed by methylation[11].

The intact procyanidin dimers B1 and B2 and the monomers catechin and epicatechin were generated during the first hour of the hydrogenolysis. This observation is most significant and is fully consistent with the chemical constitution of the polymer established in previous studies[13]. Reductive cleavage of the outer interflavanoid bond (a) would give rise to epicatechin while cleavage at bond (d) would yield catechin (see structure **3**). The procyanidin dimers may be rationalized as being produced when the inner interflavanoid linkages are broken. Thus, the breaking of bonds (b) and (c) would give rise to procyanidin dimers B2 and B1, respectively. This finding is an extension of the observation made by Haslam and co-workers[12] that during the early stages of hydrogenolysis of the procyanidin dimers both 'halves' of the dimers were detected. These results lend further corroboration to their conclusion that hydrogenolysis is an alternative method to toluene - α - thiol degradation[8] for identification of procyanidins.

As the reaction progresses, procyanidin dimers rapidly decrease in concentration and as expected, procyanidin B1 diminishes more rapidly than procyanidin B2 as the latter compound may still be generated from other portions of the procyanidin polymers. The concentration of 1 - (3, 4 - dihydroxyphenyl) - 3 - (2, 4, 6 - trihydroxyphenyl)propan - 2 - ol rises to a maximum value after *ca* 2 hr, and at this point phloroglucinol is also detected, but only as a minor product. The phloroglucinol spot becomes quite intense after the third hour and remains strong throughout the 20 hr duration of the reaction, in contrast to the 1 - (3, 4 - dihydroxyphenyl) - 3 - (2, 4, 6 - trihydroxyphenyl)propan - 2 - ol which could no longer be detected after only 4 hr. Attempts to identify the remaining two mobile spots were unsuccessful.

The major products of hydrogenolysis of the procyanidin polymer after 3 hr were not the chromatographically mobile compounds discussed above, but oligomers. These could be fractionated by chromatography of Sephadex LH-20 after the mobile components had been eluted with ethanol and the column further eluted with ethanol followed by methanol and finally 70% aqueous acetone. The IR spectra of these fractions were essentially identical to one another and similar to the spectrum of the parent *Photinia* polymer[14], suggesting that they were procyanidin in character. With the hydrogenated samples there was some increase in the intensity of absorption in the C–O stretching region (1060 cm^{-1}) and the band at 800 cm^{-1}, characteristic of the flavan - 3 - ol with the 2, 3 - *cis* - stereochemistry[15] was no longer apparent. The procyanidin character was confirmed when the samples yielded cyanidin upon reaction with hydrochloric acid in *n*-butanol. The per cent absorbances $E^{1\%}_{cm}$ of the anthocyanidin solutions measured at 546 nm and calculated on the original weight of the sample used, were of the order of 25–33% of the value of the untreated polymer which suggested that a certain proportion of the flavan moieties had been modified, so that they were no longer capable of generating anthocyanidins on acid treatment.

The ^{13}C NMR spectra of the three hydrogenated fractions were essentially similar in the aromatic carbon chemical shift region but significantly different in the aliphatic region from that of the *Photinia* polymer (Fig. 2). This indicates that epimerization has taken place, as was previously suggested by the IR data. Extra signals at δ 79.1 and 66.7 are due to C-2 and C-3 of an epicatechin chain terminating unit [2]. The relative intensities of the above signals to those of the

equivalent signals for the catechin chain terminating unit indicate that the two units are present in similar proportions. Further, extra signals at δ 83.4 and 38.1, due to C-2 and C-4 of a catechin chain extending unit [2], indicate that epimerization of the procyanidin units has also taken place. The proportion of epicatechin chain extender units is much higher (*ca* 5:1) than the catechin chain extender units, indicating that epimerization probably occurs at a much higher frequency in the terminal unit. This deduction has to be viewed with some caution because the parent polymer itself has catechin as the terminal unit which may still contribute to some extent to the high catechin ratio observed in the terminal units of these hydrogenated fractions. The presence of other ^{13}C NMR signals around δ 34, 43 and 91 which may be related to the chemical shifts of the 1, 3 - diarylpropan - 2 - ol [see Experimental for ^{13}C NMR data of 1 - (3, 4 - dimethoxyphenol) - 3 - (2, 4, 6 - trimethoxyphenyl)propan - 2 - ol)] is an indication that there are some pyran-ring opened units present. This could satisfactorily account for the lower yield of cyanidin produced from these oligomers, even after taking into consideration the fact that a smaller polymer, on a weight basis, has less procyanidin units than a large one. The hydrogenation of the aromatic A-ring could also decrease the cyanidin production, but this has not happened because the ratios of signals in the aliphatic region to the aromatic region are comparable for both the parent polymer and the hydrogenated samples.

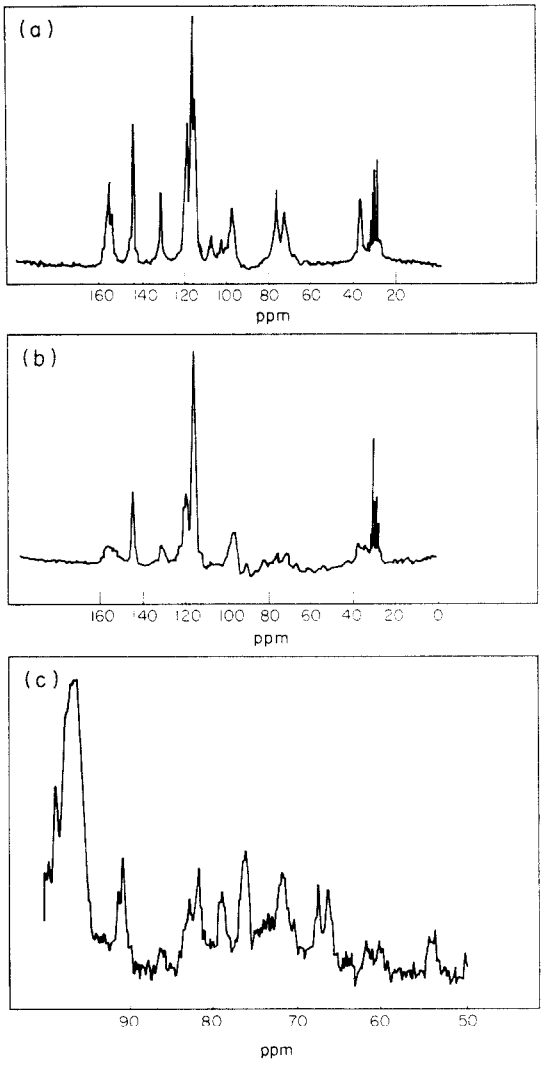


Fig. 2. ^{13}C NMR of (a) *Photinia* polymer, (b) the hydrogenated oligomer (EtOH fraction), and (c) the expanded aliphatic region of (b) measured in $(CD_3)_2CO$–D_2O (1:1).

As demonstrated in earlier studies [3] the number average MW ($\bar{M}_n$) of procyanidin polymers may be obtained from the ratio of the magnitude of the C-3 signals of the terminal flavan - 3 - ol unit (chemical shift at δ 67–68) to the extender units (chemical shift at δ 72–73). This method is not suitable for the determination of the $\bar{M}_w$ of these oligomeric fractions because of the presence of non-flavanoid units, but the ratio of the signals could still be useful in determining the approximate average number of flavan - 3 - ol units in them. In this way the number of flavan - 3 - ol units are estimated to be 2, 3 and 4–5 for fractions eluted with ethanol, methanol and 70% aqueous acetone, respectively.

The MWs of the acetates of the oligomeric fractions determined by gel permeation chromatography (GPC) on a μStyragel 10^3-Å column with tetrahydrofuran as solvent, calibrated with flavanoid and polystyrene standards, are shown in Table 1. It is most interesting to observe that chromatography on Sephadex LH-20 using ethanol, methanol and 70% aqueous acetone, in that order, as eluting solvents was strictly according to molecular size. Ethanol eluted compounds up to the size equivalent to a trimeric flavan, while methanol eluted the equivalent of a tetramer and 70% aqueous acetone eluted more polymeric materials [3, 16]. By correlating the number

Table 1. Gel permeation data of the acetates of *Photinia* proanthocyanidin polymer and the oligomeric hydrogenated fractions

Sample	$\bar{M}_n$	$\bar{M}_w$	$\bar{M}_w/\bar{M}_n$	Flavan unit equivalence
EtOH fraction	1400	1700	1.2	3
MeOH fraction	1900	2500	1.3	4
70% acetone fraction	2700	4100	1.5	5–6
Photinia polymer	5400	12000	2.2	11

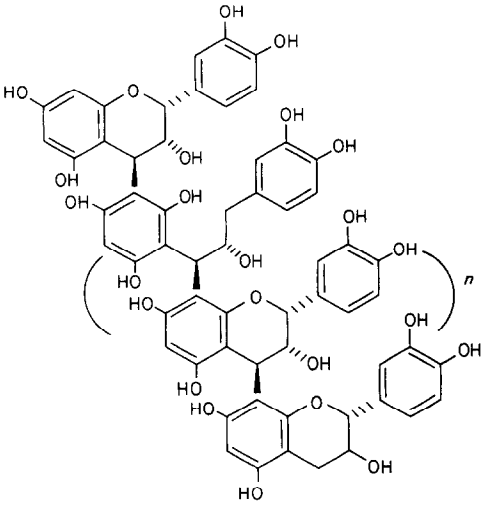
average MWs obtained from GPC and the number of flavan - 3 - ol units derived from ^{13}C NMR studies it is apparent that each oligomer contains, on average, about one ring-opened flavan - 3 - ol. This conclusion is consistent with the yields of cyanidin formed from the oligomers when related to the parent polymer. On the basis of these observations, it is reasonable to propose a structure such as **7** for these oligomers without taking into account the location of the ring-opened unit in the chain.

EXPERIMENTAL

IR spectra were measured in KBr pellets (1.5–2 mg sample in a 13-mm pellet). TLC was carried out on Schleicher & Schull cellulose on plastic sheets cut to 10 cm^2 using the solvent systems (A) *t* - BuOH–HOAc–H_2O (3:1:1) and (B) HOAc–H_2O (6:94).

Hydrogenolysis of Photinia *procyanidins.* The procyanidin polymer (2 g) in EtOH (30 ml) was hydrogenated over Pd–C (0.2 g; 10%) at 1000 psi of H_2 and 180° in a glass-lined stainless steel bomb. The reaction was performed in an upright position with no agitation of the bomb in an oven preheated to the required temp. The bomb was equipped with an outlet valve only when the progress of the reaction was to be determined. Normally the reaction was left for the desired period of time and at the end of each period the reaction mixture was cooled and worked up by filtering off the catalyst and evaporating the mixture to dryness. The residue was then partitioned between EtOAc (100 ml) and H_2O (100 ml). The aq. layer was further extracted with EtOAc (4 × 150 ml) and the extracts combined, dried (Na_2SO_4) and evapd to dryness. The % yield of the EtOAc-soluble products was obtained from the wt of the residue divided by the wt of the procyanidin polymer used in the reaction.

Procyanidin B1 and B2. The residue (0.2 g) from the EtOAc extraction of the products of a 1-hr hydrogenolysis reaction was partially purified by chromatography on a small column of Sephadex LH-20 with EtOH. The fraction containing the procyanidin dimers was examined by 2D-TLC and compared with authentic B1 and B2. The spots suspected to be the procyanidin dimers B1 and B2 cochromatographed with their respective authentic materials, B1, R_F(A) 0.38, R_F(B) 0.60 and for B2, R_F(A) 0.42, R_F(B) 0.65. The identity of the procyanidin dimers was further confirmed by co-injection with reference materials on HPLC on a Waters C18 column using 1% HOAc–MeOH (13:7) operated at a flow rate of 1 ml/min. Retention vol for B1 were 3.6 ml and for B2 4.2 ml.



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n = 0 for EtOH fraction
n = 1 for MeOH fraction
n = 2,3 for 70% aqueous acetone fraction

Phloroglucinol. The EtOAc-soluble residue (2.7 g) from two (2 × 2 g) 3-hr hydrogenolysis reactions were combined and chromatographed on Sephadex LH-20 (40 mm × 280 mm) using EtOH as eluant. The initial 200 ml was discarded and subsequent eluants were collected in 25 ml tubes. Tubes 17–30 which were shown by TLC (spots visualized by vanillin–HCl) to contain one spot, were combined and concd to yield a dark coloured gum (190 mg). Purification by prep. TLC on Si gel using $CHCl_3$–MeOH–HOAc–H_2O (85:15:10:3) gave phloroglucinol (0.05 g), R_F 0.37. On cellulose the compound had R_F(A) 0.65 and R_F(B) 0.75, ^1H NMR δ[$(CD_3)_2CO$] 5.9 (*s*) and 3.5–4.75 (*br*). The MS had a parent ion at *m/z* 126 (100%) with other major peaks at 60 (20) and 43(15).

Catechin and epicatechin. Fractions 31–57 were shown by TLC to contain the remaining chromatographically mobile vanillin–HCl active components. Evapn of the solvent yielded a gum (0.3 g) which was subjected to prep. TLC (Si gel) using $CHCl_3$–MeOH–HOAc–H_2O (85:15:10:3) to yield a complex mixture of bands. The bands at R_F 0.25 were shown by 2D-TLC on cellulose to contain mostly a mixture of catechin and epicatechin. The mixture was extracted with Me_2CO and the residue on removal of solvent was acetylated with pyridine–Ac_2O (1:1). Prep. TLC on silica gel (C_6H_6–Me_2CO, 4:1) of the acetates gave two pronounced bands at R_F 0.50 and R_F 0.45. The band at R_F 0.5 was identified as catechin pentaacetate by comparison of the chromatographic and spectroscopic data with authentic material. The MS had peaks at *m/z* at 440 (40%), 398 (95), 381 (40), 356 (100), 339 (60), 314 (100), 297 (50), 272 (95), 194 (65), 181 (70), 152 (100), 139 (100) and 123 (90); ^1H NMR δ ($CDCl_3$) 7.22 (3H, *m*), 6.68 (2H, *q*), 5.20 (2H), 2.82 (2H), 2.30 (12H), 2.20 (3H). The band at R_F 0.45 was identified as epicatechin penta-acetate by comparison with an authentic sample, The MS data were similar to those quoted for the catechin acetate; ^1H NMR: δ($CDCl_3$) 7.35(1H), 7.21(2H), 6.60(2H), 5.40(1H), 5.12(1H), 2.90(2H), 2.28(12H) and 1.92(3H).

Oligomers. Tubes 58–200 were combined and the solvent evaporated to give a residue (0.5 g). The sample gave cyanidin on treatment with 5% HCl in *n*-butanol. The $E^{1\%}_{cm}$ of the pigment generated was 43 measured at 542 nm compared to $E^{1\%}_{cm}$ 172 for the *Photinia* polymer. The acetylated compound had $\bar{M}_n$ 1400, ν_{max} 3100–3600, 1615, 1520, 1450, 1295, 1110 and 820 cm^{-1}.

The LH-20 column was then eluted with MeOH (1 l.) and the eluant concd to give a dark-coloured residue (0.07 g $\bar{M}_n$ 1900) which gave cyanidin on treatment with HCl in *n*-BuOH, $E^{1\%}_{cm}$ 60. The IR data for this material was similar to the EtOH oligomeric fraction.

The LH-20 column was finally eluted with 70% aq. Me_2CO (500 ml) and the eluate concd to give a solid (0.90 g M_n 2700). This residue yielded cyanidin on treatment with HCl in *n*-BuOH, $E^{1\%}_{cm}$ 62, and the IR data were similar to the EtOH oligomeric fraction.

1 - (3, 4 - *Dihydroxyphenyl*) - 3 - (2, 4, 6 - *trihy-*

droxyphenyl)propan - 2 - ol (**6**). Epicatechin (2 g) in EtOH (25 ml) was hydrogenated at 1000 psi of H_2 over 10% Pd on C (0.2 g) at 180° as described for the *Photinia* polymer. Examination of the mixture by 2D-TLC showed it to consist of 4 vanillin–HCl reactive products, two of which were identified as catechin and unreacted epicatechin. A sample of the reaction mixture was analysed by prep. HPLC (C-18 reverse phase column, 1% HOAc–MeOH (4:1) to yield the 1-(3, 4 - dihydroxyphenyl) - 3 - (2, 4, 6 - trihydroxyphenyl)propan - 2 - ol, R_{vol} 10 ml; MS *m/z* 292 (2%), 274 (60), 258 (12), 151 (100), 124 (80), 123 (100). This sample was identical on 2D-TLC to one of the spots from the hydrogenolysis of the *Photinia* polymer and had identical R_{vol} on HPLC to that of the combined chromatographic fractions 31–57 of the reaction product of the polymer.

Methylation of the rest of the reaction product with Me_2SO_4 in dry Me_2CO and purification by prep. TLC on Si gel (petrol–EtOAc, 1:1) gave 1 - (3, 4 - dimethoxyphenyl) - 3 - (2, 4, 6 - trimethoxyphenyl)propan - 2 - ol (0.35 g), R_F 0.35. Crystallization from MeOH gave colourless needles, mp 86–87° (lit. [11] mp 87–88°) and having the following MS *m/z* 362 (20), 211 (80), 181 (100), 167 (35), 152 (60) and ^{13}C NMR [$(CD_3)_2CO$]: 31.7 (C-3), 43.9 (C-1), 55.5–56.2 ($5 \times OCH_3$), 73.2 (C-2) and the aromatic signals at δ91.6 ($2 \times C$), 108.6, 112.9, 114.6, 122.2, 134.0, 148.6, 150.0, 160.1 ($2 \times C$) and 160.9. The sample was identical in every respect to the product derived from Na–liquid NH_3 reduction of tetramethylepicatechin followed by methylation of the generated phenol [11].

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