Condensed Tannins. Base-catalysed Reactions of Polymeric Procyanidins with Phloroglucinol: Intramolecular Rearrangements

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Reactions of polymeric procyanidins with phloroglucinol at pH 12.0 and temperatures of 23 or 50 °C gave epicatechin-(4β)-phloroglucinol (**7**), by cleavage of the interflavanoid bond between procyanidin units with subsequent addition of phloroglucinol, and (+)-catechin from the terminal unit. The phloroglucinol adduct (**7**) rearranged to an enolic form of 8-(3,4-dihydroxyphenyl)-7-hydroxy-6-(2,4,6-trihydroxyphenyl)bicyclo[3.3.1]nonane-2,4,9-trione (**9**). Rearrangement of a dimeric procyanidin phloroglucinol adduct resulted in the formation of 3'-{8-(3,4-dihydroxyphenyl)-7-hydroxy-2,4,9-trioxobicyclo[3.3.1]nonan-6-yl}-4-(3,4-dihydroxyphenyl)-2',3,4',5,6',7-hexahydroxyflavan (**10**), also in an enolic form. (+)-Catechin, from the terminal unit, gave catechinic acid, an enolic form of 6-(3,4-dihydroxyphenyl)-7-hydroxybicyclo[3.3.1]nonane-2,4,9-trione (**4**).

Condensed tannin preparations obtained by alkaline extraction of conifer barks are known to exhibit lower reactivity with aldehydes and increased acidity compared to polymeric procyanidins obtained from plants by neutral-solvent extraction.[1-3] In an effort to explain these differences, Sears *et al.*[1] refluxed (+)-catechin in aqueous sodium hydroxide and obtained two rearrangement products, catechinic acid and isocatechinic acid. Catechinic acid was shown to be an enolic form of 6-(3,4-dihydroxyphenyl)-7-hydroxybicyclo[3.3.1]-nonane-2,4,9-trione by *X*-ray crystallography of its trimethyl ether.[1] Similar structures were proposed for the reaction products of condensed tannins in alkaline solution since many of their properties were consistent with the structure of catechinic acid.[1-3] However, no products have been isolated from the reaction of condensed tannins in alkaline solutions to support this hypothesis. The base-catalysed reactions of polymeric procyanidins were examined because many of their industrial applications involve their dissolution and/or reaction at alkaline pH.[4,5]

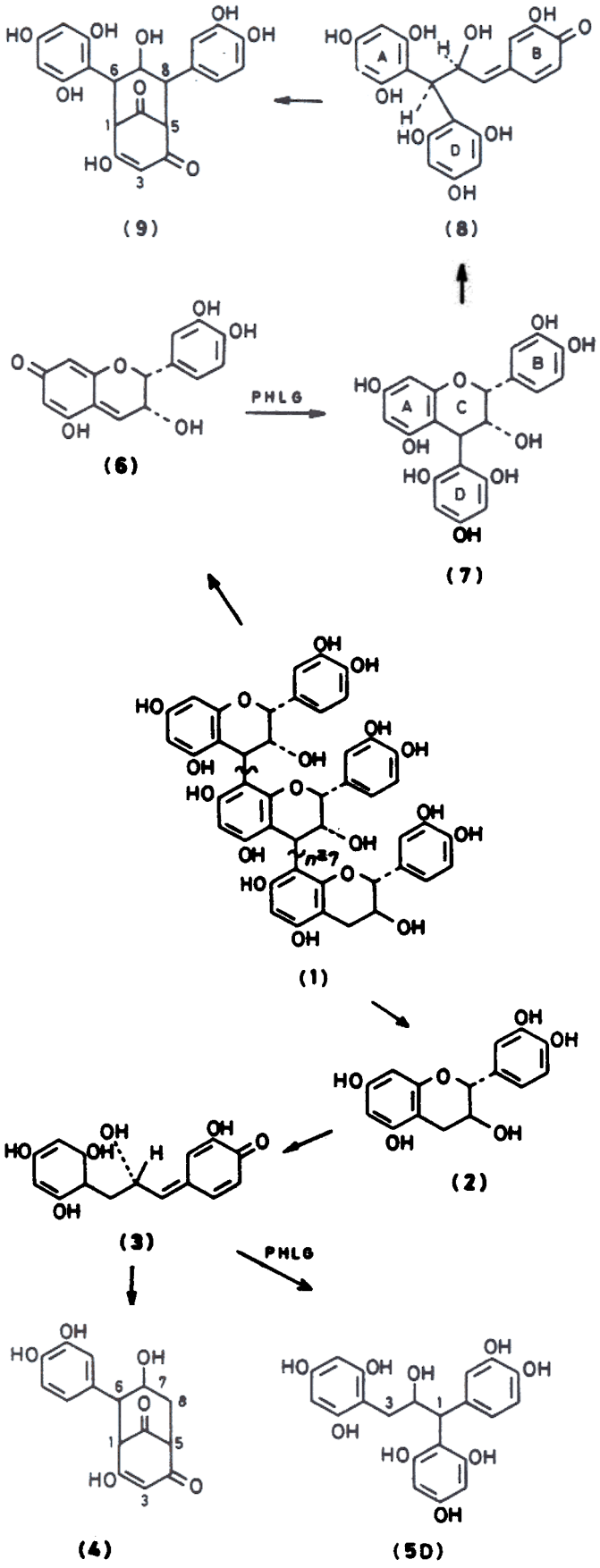
Previous studies showed that the polymeric procyanidins from loblolly pine bark[6-8] gave 1,3-dibenzylthio and 3-benzylthio derivatives of 3-(3,4-dihydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-2-ol when treated with toluene-α-thiol at pH 12.0 and ambient temperature.[9] Some diarylpropanone derivatives were also produced by loss of toluene-α-thiol and tautomeric rearrangement of the resulting quinone methide.[9] These experiments showed that the interflavanoid bond of polymeric procyanidins was more labile than the pyran ether to cleavage under alkaline conditions. To model the rearrangement reactions of condensed tannins in alkaline solutions, loblolly pine bark tannins were treated with phloroglucinol at pH 12.0 and 23 or 50 °C.

As observed in the previous study,[9] both the interflavanoid bond and the pyran ether of polymeric procyanidins (**1**) suffer rapid cleavage under these alkaline conditions (Scheme 1). Catechin (**2**), obtained from the lower terminal unit, is cleaved further within the pyran ring to give the quinone methide (**3**)† that undergoes intramolecular rearrangement to catechinic acid (**4**) as described by Sears *et al.*[1] The phloroglucinol adduct, 1-(3,4-dihydroxyphenyl)-1,3-bis(2,4,6-hydroxyphenyl)-propan-2-ol (**5D**) is not isolated from the reaction products of the polymers probably because of the comparatively low proportions of catechin terminal units (*ca.* 1 of 8 procyanidin units) and the competing rearrangement to catechinic acid (**4**).

Both (**4**) and (**5D**) are produced, however, by reaction of (+)-catechin with phloroglucinol at pH 12.0 and ambient temperature. The structure of (**5D**) is evident from fast atom bombardment mass spectroscopy (f.a.b.–m.s.), where the positive ion spectrum showed $M + 1$ at 417, and from the ^1H and ^{13}C n.m.r. spectra. Assignments of the carbon resonances at 46.8, 77.9, and 30.3 p.p.m. for the C-1, C-2, and C-3 of the propyl function; 135.8 p.p.m. for the substituted C-1 of the pyrocatechol ring; and 106.3 and 107.3 p.p.m. for the substituted C-1s of the two phloroglucinol rings are based on both proton-coupled and -decoupled spectra. The ^1H n.m.r. spectrum of the peracetate derivative of (**5D**) indicates that this compound is produced as a mixture of two isomers (1*R* and 1*S*) in approximately equal proportions. The ^{13}C n.m.r. chemical shifts of (**5D**) and related derivatives (**5A**—**C**) (Table 1) are particularly important to the elucidation of the structures of derivatives of catechinic acid described below.

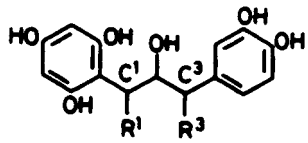
Cleavage of the interflavanoid bonds in the upper 2,3-*cis*-procyanidin units gives a quinone methide (**6**) that reacts stereospecifically with phloroglucinol to give epicatechin-(4β)-phloroglucinol (**7**). The stereospecificity of this reaction was also observed in the acid-catalysed cleavage of polymeric 2,3-*cis*-procyanidins in the presence of an excess of phloroglucinol.[6,10] However, the yield of (**7**) is lower under alkaline conditions because it is readily converted, through the quinone methide (**8**), into the major product isolated, an enolic form of 8-(3,4-dihydroxyphenyl)-7-hydroxy-6-(2,4,6-trihydroxyphenyl)bicyclo[3.3.1]nonane-2,4,9-trione (**9**). The structure of (**9**) is evident from fast atom bombardment mass spectroscopy ($M + 1 = 415$), and from ^1H and ^{13}C n.m.r. spectroscopy. Comparison of proton-coupled and -decoupled ^{13}C n.m.r. spectra with the spectral features of (**4**) (Table 3) and other model compounds (Tables 1 and 2) permits assignment of most carbon resonances. Signals at 37.2, 71.2, and 45.2 p.p.m. are assigned to C-6, C-7, and C-8 of the bicyclic ring, respectively. Signals at 50.7 and 61.5 p.p.m. due to C-5 and C-1 of this ring are assigned on the basis of a HETCORR C–H connectivity experiment and comparison with the spectrum of catechinic

† Intermediates (**3**), (**6**), (**8**), and (**12**)—(**16**) are postulated and have not been isolated.



Scheme 1. Proposed routes to the formation of compounds (4), (5D), and (9) oy reaction of procyanidins with phloroglucinol (PHLG)

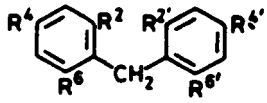
Table 1. ^{13}C N.m.r. chemical shifts of methine carbons related to those in compounds (4), (9), and (10)



Compd.	R^1	R^3	Chemical shift[a] C-1	C-2	C-3	Change C-1	C-2	C-3
(5A)	H	H	31.6	75.8	44.2			
(5B)	H	SCH_2Ph	30.4	76.9	56.9	−1.2	+1.1	+12.7
(5C)	SCH_2Ph	SCH_2Ph	48.6	79.2	55.0	+17.0	+3.4	+10.8
(5D)	H	Phloro	30.3	77.9	46.8	−1.3	−1.3	+2.6

[a] Recorded at 20 MHz at *ca.* 35 °C in [$^2H_6$]acetone.

Table 2. ^{13}C N.m.r. chemical shifts of benzyl methylenes related to compounds (4), (9), and (10)



Compd.	R^2	R^4	R^6	$R^{2'}$	$R^{4'}$	$R^{6'}$	Chemical shift[a]	Change
(11A)	H	OH	H	H	OH	H	40.6	
(11B)	OH	H	H	OH	H	H	30.0	−10.6
(11C)	OH	OH	H	OH	OH	H	29.0	−11.6
(11D)	OH	OH	OH	H	OH	H	31.0	−9.6
(11E)	OH	OH	OH	OH	OH	OH	17.0	−23.6

[a] Recorded at 20 MHz at *ca.* 35 °C in [$^2H_6$]acetone.

acid (4) (Table 3). An enolic form of the bicyclic ring is evident from a resonance at 106.5 p.p.m., assigned to C-3 and from signals at 188 p.p.m. (C-2 + 4) and at 206 p.p.m., assigned to the unconjugated carbonyl at C-9. Resonances at 155—157 p.p.m., 104.8 and 101.2, as well as at 96.3 and 97.8 indicate one phloroglucinol ring but in two different environments. This can be due to either restricted rotation or to the presence of two different isomers. Chemical shifts for the pyrocatechol ring are similar to those observed for this ring in the spectrum of catechinic acid (4).

An ^1H n.m.r. spectrum of the acid form of (9) recorded at 250 MHz in [$^2H_6$]acetone–water shows the two protons in the phloroglucinol ring at 6.02 and 6.05 p.p.m. each with coupling constants of *ca.* 2.2 Hz, consistent with a phloroglucinol ring that does not rotate [compare phloroglucinol ring protons in (+)-catechin].[10] In [$^2H_6$]acetone or in [$^2H_4$]methanol these proton signals converge to a singlet. The 3-H proton of the bicyclic ring is most clearly seen in the spectrum recorded in [$^2H_6$]acetone where it is a singlet at 5.55 p.p.m. This signal is absent in spectra recorded with ^2H_2O present or as the sodium salt. The 7-H signal is a double doublet centred at 4.61 p.p.m. with coupling constants of 10.8 and 4.7 Hz. Therefore, one of the aryl functions is axial and the other is equatorial. The other protons of the bicyclic ring system are shown more clearly in a spectrum of the sodium salt recorded in [$^2H_4$]methanol. Here the broad OH signal is shifted downfield to *ca.* 4.9 p.p.m. and the 3-H signal is not evident. In this solvent, the 7-H signal appeared at 4.66 p.p.m. with coupling constants of 11.0 and 5.0 Hz. A 1 H double doublet centred at 3.75 p.p.m. has coupling constants of 5.0 and 3.6 Hz. Another 1 H double doublet centred at 2.87 p.p.m. has coupling constants of 11.0 and 4.6 Hz. The HETCORR experiment established that the proton at δ_H 3.75

Table 3. ^{13}C N.m.r. chemical shifts for catechinic acid and its phloroglucinol and flavan adducts

Compd.	Ring	Carbon number[a] 1	2	3	4	5	6	7	8	9
(4)	Bicyclic	59.2	211	106.5	192	64.2	54.5	67.3	37.7	211
	Pyrocatechol	132.5	116	144.8	145	117.4	122			
(9)	Bicyclic	61.5	206	106.4	188	50.7	37.2	71.2	45.6	206
	Pyrocatechol	131.7	116	145.0	145	117.4	122			
	Phloroglucinol	101.2	155—157	96.0	159	96.0	155—157			
		(104.8)		(97.8)		(97.8)				
(10)	Bicyclic (F)	61.3	206	106	188	50.7	36.7	70.4	45.5	206
	Pyrocatechol (B)	136.3	116	145	145	117.8	120.9			
	Pyrocatechol (E)	131.4	116	145	145	117.6	121.6			
	Phloro (A)	99.7	157	97.1	157	95.5	157			
	Phloro (D)	101.2	157	116.0	157	95.7	157			
	Pyran (C)		67.9	73.1	45.6					

[a] Note that the pyrocatechol ring in (4) is at C-6 but at C-8 in the products (9) and (10) (Figure). Spectra were recorded at 20 MHz at *ca.* 35 °C in [$^2H_6$]acetone + ^2H_2O (1:1, v/v).

was attached to a carbon with a chemical shift of δ_C 37.2 and that the proton at δ_H 2.87 was attached to a carbon with a chemical shift of δ_C 45.6 p.p.m. Two, 1 H multiplets, centred at 2.70 and 2.55 p.p.m., can be interpreted as pairs of doublets of approximately equal proportion; one pair at 2.70 and 2.69 p.p.m. each with coupling constants of 4.6 Hz, and the other at 2.56 and 2.55 p.p.m. each with coupling constants of 3.6 Hz. Doubling of the 1-H and 5-H signals is expected from tautomerism of the enol and ketone functions. Methylation of (9) gave two hexamethyl ether derivatives in approximately equal proportions, both of which have the molecular formula $C_{27}H_{30}O_9$. Although two stereoisomers are present, the proton spectra show that the aryl functions at C-6 and C-8 exist in only one configuration relative to the 7-hydroxy group since these proton signals are sharp and clearly first-order. A second-order proton spectrum is evident only in the 1-H and 5-H signals of the bicyclic ring.

If it is assumed that (9) is produced by rearrangement of epicatechin-(4β)-phloroglucinol (7), the formation of a product with one configuration of the aryl substituents at C-6 and C-8 and coupling constants consistent with the above results can be accounted for in two ways. One isomer results from attack of the phloroglucinol ring D on the quinone methide (8) stereospecifically to the side opposite that of the aliphatic hydroxy group in (7). The product of this reaction has the phloroglucinol ring in an axial position with the 7-hydroxy group and the pyrocatechol ring on C-8 in equatorial positions, accounting for the 7-H coupling constants as $J_{7,8}$ 11.0 and $J_{6,7}$ 5.0 Hz. The COSY experiment permits assignment of 8-H to the signal at 2.87 p.p.m. and 6-H to the signal at 3.75 p.p.m. This, together with 5-H at 2.55 with $J_{5,6}$ 3.6 Hz and 1-H at 2.70 p.p.m. with $J_{1,8}$ 4.6 Hz, requires the enolic portion of the nonane ring to be below the plane of the cyclohexanone ring system, that is in a chair conformation [Figure, (9A)].

The other isomer [Figure, (9B)] that would have similar proton coupling constants, could also be formed by cleavage within the pyran ring of (7) to give the quinone methide (8). However, in this case the phloroglucinol A ring would have to attack the quinone methide from the same side as the aliphatic hydroxy group (Scheme 1). This isomer has the pyrocatechol ring in an axial position and the 7-hydroxy group and the phloroglucinol ring at C-6 in equatorial positions. As with (9A), the enolic portion of the bicyclic ring is below the plane of the cyclohexanone ring system. Structure (9B) requires a reversal of the assignments described above for structure (9A) for both 1-H and 5-H and 6-H and 8-H.

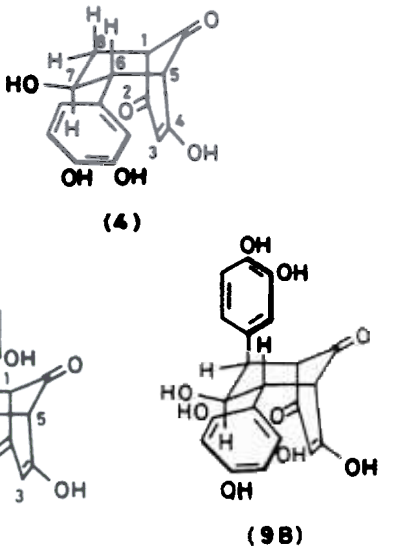


Figure. Structure of catechinic acid (4) and alternative structures (9A) and (9B) for the major product of procyanidins with phloroglucinol

A Dreiding model of (8) suggests that its further reaction can occur by approximately equal attack of either phloroglucinol ring on the quinone methide, but from opposite sides, giving a mixture of (9A) and (9B). However, as discussed above, the ^1H n.m.r. spectra indicate that only one configuration of the two aryl groups at C-6 and C-8 is formed. The Dreiding model shows that the nucleophilic carbons of the D ring would be in a favourable position to attack the quinone methide from the side opposite the aliphatic hydroxy group as soon as the pyran ring opens into intermediate (8) and prior to its full conformational unfolding. This mechanism would yield a single isomer, (9A), consistent with the ^1H n.m.r. data.

The decoupled ^{13}C (Table 3) and HETCORR spectra of (9) also indicate structure (9A). Whether structure (9A) or (9B) is correct, the chemical shift assignments (Table 3) for all the carbons would be approximately the same, except for the pairs C-6 and C-5 and C-8 and C-1. The chemical shifts of C-6 and C-8 and their associated proton coupling constants are important indicators of which structure is correct.

For structure (9A), the chemical shift of C-8 can be calculated

by substracting *ca.* 6 p.p.m. (approximate gamma gauche effect[11,12] of the axial phloroglucinol) from the chemical shift observed for C-6 of compound (**4**) (Table 3). This gives a calculated value of 48.5 p.p.m. for the chemical shift of C-8 in structure (**9A**) compared to the signal at 45.6 p.p.m. in the spectrum of (**9**). The signal at 37.2 p.p.m. can then be assigned to C-6. This assignment is consistent with the effect of *ortho*-hydroxylation on the chemical shift of benzyl methylenes (Table 2).[9-13,14] In addition, the assignment is consistent with chemical shifts of C-1 and C-3 in the spectra of C-3 benzylthio, phloroglucinol, and 1,3-dibenzylthio adducts formed on reaction of polymeric procyanidins with these nucleophiles (Table 1).[9] The chemical shift of C-3 in the diphenylpropan-2-ol (**5A**) is not very different from that of (**5D**) in which a hydrogen has been substituted with a phloroglucinol ring. Therefore, the chemical shift of C-6 in structure (**9A**) should be essentially unchanged from that of C-8 in compound (**4**) as was observed (37.2 compared to 37.7 respectively) (Table 3).

For structure (**9B**), the chemical shift of C-6 can be calculated using the chemical shift for C-6 of (**4**) (Figure) as a reference when there is an equatorial pyrocatechol ring at C-6. In structure (**9B**), the phloroglucinol ring at C-6 is equatorial. Thus, the chemical shift of C-6 in (**9B**) is equal to *ca.* 54.5 p.p.m. [the chemical shift of C-6 in (**4**)] minus 10 to 12.6 p.p.m. [the electronic effect of changing the hydroxylation pattern of an aromatic substituent from that of pyrocatechol to that of phloroglucinol (Table 2) and references[13,14]] minus 6 p.p.m. (approximate gamma gauche effect[11,12] of the axial pyrocatechol ring). This gives a calculated value of 35.9—38.5 p.p.m. for the chemical shift of C-6 in structure (**9B**). Therefore, the signal at 37.2 p.p.m. in the spectrum of (**9**) can be assigned to C-6 and the signal at 45.6 p.p.m. to C-8 of structure (**9B**). Thus, carbons carrying phloroglucinol and pyrocatechol rings are expected to resonate in the regions of 37 and 46 p.p.m. respectively in either structure (**9A**) or (**9B**). The HETCORR experiment shows that the proton at 3.75 p.p.m. correlates with the carbon signal at 37.2 p.p.m. and the proton at 2.87 p.p.m. correlates with the carbon signal at 45.6 p.p.m. The coupling constants for these proton signals are only consistent with an axial substituent at C-6 and an equatorial substituent at C-8 or the structure (**9A**) (Figure).

A further product, isolated from the reaction of loblolly pine bark tannins with phloroglucinol at pH 12.0 and 23 °C, is tentatively identified as 3′-{8-(3,4-dihydroxyphenyl)-7-hydroxy-2,4,9-trioxobicyclo[3.3.1]nonan-6-yl}-4-(3,4-dihydroxyphenyl)-2′,3,4′,5,6′,7-hexahydroxyflavan [Scheme 2, (**10**)]. The structure of (**10**) is evident from fast atom bombardment mass spectroscopy ($M + 1 = 715$), and from the ^{1}H and ^{13}C n.m.r. spectra (Table 3). The ^{13}C spectrum shows signals at approximately the same chemical shifts as were observed in the spectrum of (**9**). In addition, resonances occur at chemical shifts indicative of a further phloroglucinol ring, a further pyrocatechol ring, and a pyran ring similar to the C-4 substituted flavan-3-ols.

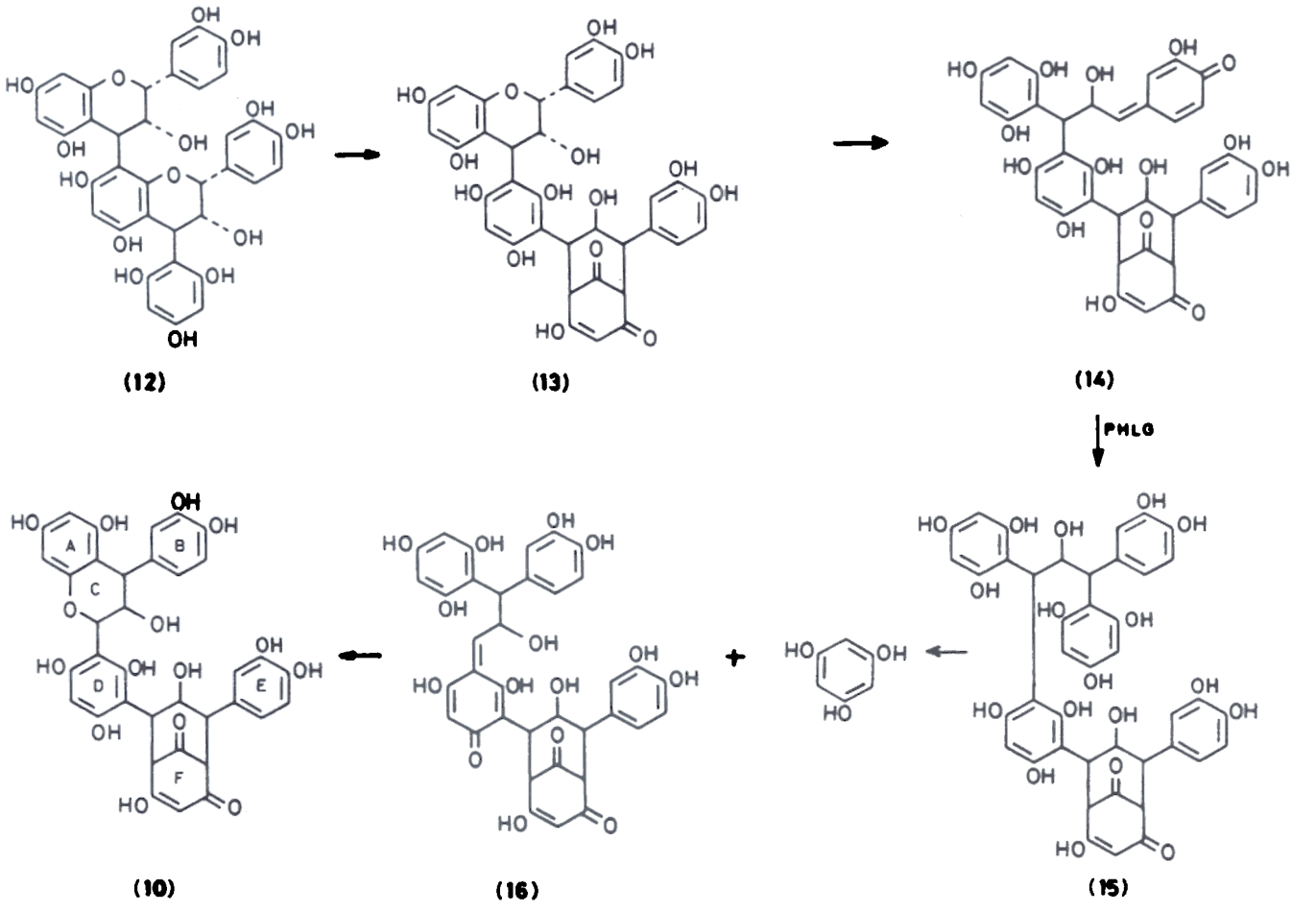
Both the stereochemistry and substitution of the pyran ring-C in (**10**) are evident from the chemical shifts of the carbons in this heterocyclic ring. The signal at 73.1 p.p.m. is assigned to C-3 since this carbon is not expected to be influenced greatly by changes in stereochemistry or substitution at C-2 or C-4 [compare chemical shifts of 72.9 and 72.6 p.p.m. for C-3 in catechin-(4α)-phloroglucinol and epicatechin-(4β)-phloroglucinol (**7**)[15] and 70.3 for C-3 in epicatechin-(4β)-resorcinol[16]]. The signal at 67.9 is then assigned to C-2 on the basis of an expected upfield shift of *ca.* 10 p.p.m. due to replacement of the pyrocatechol ring at C-2 by a phloroglucinol derivative where two hydroxy groups are *ortho* to the methine carbon [compare methylene carbon chemical shifts in (**11A—E**), Table 2].[13,14] The large upfield shift of the C-2 carbon to 67.9 is also consistent with a 2,3-*cis*-3,4-*trans* stereochemistry [compare C-2 chemical shifts of 76.6 and 83.8 for epicatechin-(4β)-phloroglucinol (**7**) and catechin-(4α)-phloroglucinol respectively].

Similar conclusions are reached on the basis of changes in the chemical shift of C-4 due to substitution of the heterocyclic ring in (**10**). The C-4 of epicatechin-(4β)-phloroglucinol appears at 36.5 p.p.m.[15] and that of epicatechin-(4β)-resorcinol appears at 38.7 p.p.m.[16] Replacement of the phloroglucinol ring at C-4 with a pyrocatechol ring can be expected to cause a downfield shift of *ca.* 10 p.p.m. (Table 2); the chemical shift of C-4 in (**10**) occurs at 45.6 p.p.m. This assignment is consistent with the chemical shift of 46.8 p.p.m. observed for the methine carbon in (**5A**). Other important features of the carbon spectrum of (**10**) supporting these conclusions include: (*a*) the downfield shift of the substituted C-1 of the pyrocatechol ring B to 136.3 p.p.m. [compare a chemical shift of 135.8 p.p.m. for the substituted C-1 of the pyrocatechol ring in (**5A**) with 132.2 p.p.m. for this carbon in (**7**)], and (*b*) the signal at 116 p.p.m. assigned to the substituted phloroglucinol carbon on ring D on the basis of increased deshielding associated with substitution at C-2 of the pyran ring C [compare a chemical shift of *ca.* 107.3 p.p.m. for the substituted phloroglucinol carbons in (**5**) and (**7**)].

An ^{1}H n.m.r. spectrum of (**10**) recorded at 250 MHz in [$^2H_4$]methanol shows protons with chemical shifts and coupling constants essentially identical with those described for compound (**9**). In addition, the spectrum contains a 1 H singlet at 5.30 p.p.m. assigned to 2-H, a 1 H doublet with $J_{3,4}$ 1 Hz at 4.09 p.p.m. assigned to 4-H, and a 1 H doublet with $J_{3,4}$ 1 Hz at 4.19 p.p.m. assigned to 3-H of the pyran ring C. The stereochemistry of the flavan ring system must, therefore, be 2,3-*cis*-3,4-*trans*. The $J_{3,4}$ coupling would be expected to be *ca.* 5 Hz if the stereochemistry was 2,3-*cis*-3,4-*cis*.[17] Consideration of plausible mechanisms together with Dreiding models suggest that (**10**) has a 2*S*,3*S*,4*R* absolute stereochemistry in the pyran ring system of the upper unit. Roux and co-workers[18,19] have recently described similar rearrangement products in phlobatannins from *Acacia* and have demonstrated analogous base-catalysed rearrangement of profisetinidins.

The reactions of polymeric procyanidins with phloroglucinol at pH 12.0 and ambient temperature are summarised in Schemes 1 and 2. Catechin, liberated from the terminal unit, is cleaved within the pyran ring to give a quinone methide (**3**) that undergoes intramolecular condensation and rearrangement to catechinic acid (**4**). Cleavage of the interflavanoid bonds gives a quinone methide (**6**) from the upper units that reacts initially with phloroglucinol to give epicatechin-(4β)-phloroglucinol (**7**). The isolation of (**7**) and absence of a di-phloroglucinol adduct analogous to (**5**) suggests that cleavage of the interflavanoid bond is more facile than cleavage within the heterocyclic ring; this conclusion is also consistent with the formation of predominantly one isomer at C-1 in the dibenzylthio derivatives obtained from the reaction of tannins with toluene-α-thiol at pH 12.0.[9] Epicatechin-(4β)-phloroglucinol is obtained in low yield and only under mild reaction conditions. It reacts by cleavage within the pyran ring to give the quinone methide (**8**) that undergoes an intramolecular condensation and rearrangement similar to the formation of catechinic acid that leads to the 6-phloroglucinol adduct (**9**). The formation of the isomer (**9A**) indicates a stereospecific reaction of the phloroglucinol ring D on the quinone methide from the side opposite the aliphatic hydroxy group.

Random cleavage of the interflavanoid bonds within the polymer generates some epicatechin-(4β→8)-epicatechin-(4β)-phloroglucinol [Scheme 2, (**12**)].[6,10] The lower flavan unit of (**12**) is cleaved within the pyran ring with a rearrangement analogous to the formation of catechinic acid (**4**) and the phloroglucinol adduct (**9**) to give (**13**). The pyran ring of the upper flavan unit is also cleaved to give a quinone method intermediate (**14**). Here the bulk of the lower flavan unit inhibits



Scheme 2. Proposed route to the formation of compound (10): PHLG = phloroglucinol

intramolecular condensation so that (14) reacts with phloroglucinol to give the intermediate (15). Previous work has shown that a methine which is substituted with two phloroglucinol functions eliminates phloroglucinol readily.[20] The quinone methide (16) produced from loss of phloroglucinol then reacts stereospecifically with the hydroxy group of the remaining phloroglucinol function to produce a pyran ring in a reaction analogous to the epimerisation of flavan-3-ols in alkaline solution.

The above rearrangements have proved to serve as useful models for interpretation of the reactions of polymeric procyanidins in alkaline solution without the addition of an external nucleophile. Essentially all of the major ^{13}C n.m.r. resonances observed in the products of reaction of loblolly pine bark tannins at pH 12.0 and 23—50 °C can be accounted for by analogy to the spectral properties of (4), (9), and (10).[21] These results help to explain the low aldehyde reactivity, and acidity of polymeric procyanidins that have been extracted from plant tissue or reacted at alkaline pH. Although these reactions are harmful to the reactivity of condensed tannins extractable from conifer tree barks in applications such as their use in wood adhesives, alkaline solutions can be used, even in cold-setting phenolic adhesive systems, when these rearrangement reactions are properly controlled.[4,5]

Experimental

^{1}H and ^{13}C N.m.r. spectra were recorded using either a Varian TF-80A or a Bruker WM-250.* COSY and HETCORR experiments[22] were carried out with the Bruker WM-250 through the courtesy of Dr. L. L. Landucci, Forest Products Laboratory, USDA-Forest Service, Madison, Wisconsin. Optical rotations were measured with a Jasco DIP-181 polarimeter. Fast-atom bombardment and high-resolution electron-impact mass spectral determinations were performed by the Midwest Centre of Mass Spectrometry, a National Science Foundation Regional Instrumentation Facility (Grant No. CHE 8211164). Elemental analyses were performed by Galbraith Laboratories Inc., Knoxville, Tennessee. F.t.-i.r. spectra were provided by Dr. T. P. Schultz, Mississippi State University, Mississippi State, Mississippi. Column chromatography was on Sephadex LH-20 with either ethanol or ethanol–water (1:1, v/v) solvents. Thin-layer chromatography was on either Schleicher and Shuell F1440 or Baker-flex F cellulose plates developed with 6% acetic acid and/or TBA, t-butyl alcohol–acetic acid–water (3:1:1, v/v/v). Plates were visualised by spraying with vanillin–HCl. Condensed tannins were isolated from the phloem of loblolly pine trees as has been described previously.[9] Phloroglucinol and other reagents were used as purchased. An authentic sample of catechinic acid was kindly provided by Dr. K. D. Sears, ITT-Rayonier Inc., Shelton, Washington.

Alkaline Reaction of Procyanidins with Phloroglucinol at 50 °C for 24 h.—Purified loblolly pine bark tannins (5.0 g) were combined with phloroglucinol (5.0 g) in deoxygenated water (80 ml). Solid NaOH was used to adjust the pH to 12.0 with

* Mention of trade names does not constitute endorsement by the USDA-Forest Service.

constant magnetic stirring under a nitrogen atmosphere. The solution was sealed in a 100 ml reaction vial and kept in a 50 °C water-bath for 24 h. The pH was adjusted to 7.0 with dilute HCl and the solution extracted with ethyl acetate (4 × 30 ml portions) to remove the excess of phloroglucinol. The aqueous phase was freeze-dried and then applied to a Sephadex LH-20 column (2.5 × 75 cm) packed in and eluted with 95% ethanol. Elution of fractions (15 ml) was monitored by one-dimensional cellulose t.l.c. developed with 6% acetic acid.

8-(3,4-*Dihydroxyphenyl*)-7-*hydroxy*-6-(2,4,6-*trihydroxyphenyl*)*bicyclo*[3.3.1]*nonane*-2,4,9-*trione* (**9**) in an enolic form was isolated as the sodium salt from fractions 83—160 eluted with 95% ethanol from a Sephadex LH-20 column. After the salt had passed through an Amberlite IR-120 (H+ form) ion-exchange resin column, the acid form was isolated from fractions 86—165 in a second Sephadex LH-20 purification. It was recovered by freeze-drying as a light brown amorphous solid (Found: C, 50.65; H, 5.3. $C_{21}H_{18}O_9$·4.8H_2O requires C, 50.36; H, 5.52); f.a.b.-m.s. showed M + 1 415 ($C_{21}H_{18}O_9$ + 1 requires 415). The product had R_F values of 0.81 and 0.58 on cellulose t.l.c. plates developed with 6% acetic acid and TBA respectively. When the plates were sprayed with vanillin–HCl, the compound turned red and then slowly changed to an intense yellow. The ^{13}C n.m.r. spectrum is described in Table 3; the ^1H n.m.r. spectrum recorded at 250 MHz in [$^2H_4$]methanol showed δ (from TMS) 2.55—2.57 (1 H, d, J 3.6 Hz, 5-H), 2.68—2.70 (1 H, d, J 4.6 Hz, 1-H), 2.87 (1 H, dd, J 4.6 and 11.0 Hz, 8-H), 3.75 (1 H, dd, J 3.6 and 5.0 Hz, 6-H), 4.66 (1 H, dd, J 5.0 and 11.0 Hz, 7-H), 5.96 (2 H, s, phloroglucinol ArH), 6.45—6.64 (3 H, m, pyrocatechol ArH); ^1H n.m.r. recorded in [$^2H_6$]acetone showed δ (from TMS) 2.69 (1 H, m, 5-H), 2.89 (1 H, m, 1-H), 2.98 (1 H, dd, J 4.5 and 10.8, 8-H), 3.71 (1 H, dd, J 3.8 and 4.7 Hz, 6-H), 4.61 (1 H, dd, J 4.7 and 10.8 Hz, 7-H), 5.55 (1 H, s, 3-H), 6.02 (2 H, s, phloroglucinol ArH), and 6.45—6.7 (3 H, m, pyrocatechol ArH). Proton-proton couplings were established by COSY and proton–carbon connectivities by HETCORR experiments. F.t.-i.r. showed carbonyl absorbance at 1 730 cm^{-1}.

Methylation of compound (**9**) with dimethyl sulphate in acetone over potassium carbonate gave two products in approximately equal proportions as determined by t.l.c. (silica gel; benzene–ethanol–water–acetic acid, 200:47:15:1, v/v/v/v, upper phase). The two predominant products had R_F values of 0.68 and 0.58. Preparative t.l.c. gave the compound at R_F 0.68 as a white amorphous solid after precipitation from n-hexane; $[\alpha]_{589}$ −268° (*c* 0.06, $CHCl_3$). E.i.-m.s. showed a parent ion 498.1891 (49.3%) for $C_{27}H_{30}O_9$ as required for the expected hexamethyl ether derivative. The compound at R_F 0.58 was also recovered as a white amorphous solid after precipitation from n-nexane; $[\alpha]_{589}$ −142° (*c* 0.14, $CHCl_3$). E.i.-m.s. showed a parent ion 498.1882 (100%) for $C_{27}H_{30}O_9$ also consistent with a hexamethyl ether derivative.

6-(3,4-*Dihydroxyphenyl*)-7-*hydroxybicyclo*[3.3.1]*nonane*-2,4,9-*trione* (**4**) was isolated in an enolic form as the sodium salt from fractions 74—85. It was identified by comparison of chromatographic and spectral properties with those of authentic catechinic acid. It had R_F values of 0.55 and 0.50 on cellulose t.l.c. plates developed with 6% acetic acid and TBA. Like compound (**9**), when sprayed with vanillin–HCl it also turned red and changed to an intense yellow. The ^{13}C n.m.r. spectral data for this compound are summarised in Table 3.

Alkaline Reaction of Procyanidins with Phloroglucinol at 23 °C *for* 18 h.—The reaction conditions previously described were used except that the reaction was kept at room temperature for 18 h. Compounds (**4**) and (**9**) were observed by two-dimensional cellulose t.l.c. but were not collected. Fractions 101—117 were collected and evaporated to give 0.66 g of an amorphous brown solid. This was applied to a further Sephadex LH-20 column (1.5 × 80 cm) packed and eluted with 95% ethanol.

3′-{8-(3,4-*Dihydroxyphenyl*)-7-*hydroxy*-2,4,9-*trioxobicyclo*[3.3.1]*nonan*-6-*yl*}-4-(3,4-*dihydroxyphenyl*-2′,3,4′,5,6′,7-*hexahydroxyflavan* (**10**) was obtained in the sodium salt form as a brown amorphous solid (64 mg) from the combined fractions 44—57. It had R_F values of 0.74 and 0.61 on cellulose t.l.c. plates developed with 6% acetic acid and TBA. When plates were sprayed with vanillin–HCl, (**10**) also went red and later changed to yellow. The compound was obtained in the acid form by passing it through an Amberlite IR-120 column. F.a.b.-m.s. showed M + 1 715 ($C_{36}H_{30}O_{15}$ requires 715). The ^{13}C n.m.r. spectrum is summarised in Table 3; the ^1H n.m.r. spectrum recorded at 250 MHz in [$^2H_4$]methanol showed δ (from TMS) 2.74 (1 H, m, 5-H ring F), 2.84 (1 H, m, 1-H ring F), 2.92 (1 H, dd, J 5.0 and 11.0 Hz, 8-H ring F), 3.76 (1 H, dd, J 4.0 and 4.5 Hz, 6-H ring F), 4.09 (1 H, d, J 1.0 Hz, 4-H ring C), 4.19 (1 H, d, J 1.0 Hz, 3-H ring C), 5.31 (1 H, s, 2-H ring C), 5.98 (2.5 H, m, ArH, rings A and D), and 6.5—6.7 (6 H, m, ArH rings B and E). The phloroglucinol ring protons were partially exchanged. The enolic proton (3-H of ring F) was completely exchanged. The 7-H proton of ring F was obscured by a large OH signal.

Reaction of Catechin with Phloroglucinol at pH 12.0 *and* 23 °C.—(+)-Catechin (2 g) as received from Fluka was combined with phloroglucinol (4 g) in deoxygenated water (40 ml) and the pH adjusted to 12.0 by addition of solid NaOH. The solution was sealed in a reaction vial and kept at ambient temperature for 16 h. The solution was acidified to pH 7.0 and extracted with ethyl acetate to give a brown amorphous solid. The product was applied to a Sephadex LH-20 column (2.5 mm × 80 cm) packed in and eluted with 95% ethanol. Fractions 30—50 were combined to afford a tan solid (440 mg) which appeared to be predominantly one compound by t.l.c. The combined fractions were rechromatographed on a Sephadex LH-20 column (1.5 × 80 cm).

1-(3,4-*Dihydroxyphenyl*)-1,3-*bis*-(2,4,6-*trihydroxyphenyl*)*propan*-2-*ol* (**5D**). Fractions 46—56 were combined to give a light-tan solid; f.a.b.-m.s. showed M + 1 417 ($C_{21}H_{20}O_9$ + 1 requires 417); the ^{13}C n.m.r. spectrum showed δ (from acetone) 30.3, 30.7 (propyl C-3), 46.8 (propyl C-1), 77.9 (propyl C-2), 96.8 and 96.9 (phloroglucinol C-3 and C-5), 106.1 (phlorolgucinol C-1), 107.3 (phloroglucinol C-1), 116.1 (pyrocatechol C-2), 117.9 (pyrocatechol C-5), 121.0 (pyrocatechol C-6), 135.8 (pyrocatechol C-1), 144.2 and 145.8 (pyrocatechol C-3 + C-4), and 158.2—158.9 (phloroglucinol C-2, C-4, and C-6).

Acetylation · (acetic anhydride–pyridine) gave a white amorphous solid (Found: C, 58.4; H, 4.9. $C_{39}H_{38}O_{18}$ requires C, 58.9; H, 4.8%); the ^1H n.m.r. spectrum of the peracetate showed δ (from TMS) 2.10 and 2.22 (aliphatic and aromatic OAc), 3.02 and 3.06 (0.5 H each, J 5.0 Hz, propyl 1-H), 4.76 (2 H, d, J 9.7 Hz, propyl 3-H), 6.01 (1 H, m, J 9.7 and 5.0 Hz, propyl 2-H).

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