Phytochemistry. 1980. Vol. 19, pp. 1747–1754. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0801-1747 $02.00/0

THE PHYTOCHEMISTRY OF PROANTHOCYANIDIN POLYMERS

LAI YEAP FOO and LAWRENCE J. PORTER

Chemistry Division, Department of Scientific and Industrial Research, Petone, New Zealand

(*Received* 31 *October* 1979)

Key Word Index – Proanthocyanidin polymers; condensed and hydrolysable tannins; structures; phytochemical significance.

Abstract—The structures of 38 proanthocyanidin polymers (condensed tannins) from 14 widely distributed families of plants are described. The polymers have been isolated from a wide variety of tissues including fruit (ripe and unripe), leaves, bark and phloem. They are all based on a common 4–8 (or 6) linked polyflavan-3-ol structure, analogous to B-type proanthocyanidin dimers.

INTRODUCTION

Since the early discovery by the Robinsons [1, 2] of the wide distribution of proanthocyanidins in plants, and the later generalization by Bate-Smith [3, 4] that these compounds appeared to be largely concentrated in plants of a woody habit, their presence, and the type of anthocyanidin yielded on acid treatment have been commonly used as a phytochemical character. There have been various attempts to refine these observations. Weinges [5–7], Haslam [8–10] and their associates have concentrated on the structure of the procyanidin dimers and trimers but have not attempted to draw taxonomic inferences from these observations. In contrast Bate-Smith has concentrated largely on the predominating proanthocyanidin polymers and has used their solubility and protein-precipitating ability [11] (degree of astringency) in an attempt to shed light on the taxonomic significance of proanthocyanidin distribution [12].

The latter approach would seem to be based on sounder principles than study of the dimers, as the major biosynthetic thrust is directed towards polymer synthesis in plants. Moreover, if proanthocyanidins have a defence or physiological function in plants, it is more likely to be associated with the polymer fraction, which is known to bind protein [11, 13, 14].

Our recent development of the methodology [15] to readily isolate, purify and accurately determine the basic structural features of proanthocyanidin polymers (e.g. the average B-ring oxidation pattern, heterocyclic ring stereochemistry of the constituent flavan-3-ol units, the nature of the chain-terminating unit and, in favourable cases, the number average molecular weight (M_n)) affords a new approach to the systematic study of proanthocyanidin polymers.* The current survey, although not extensive, is sufficient to appraise the scope and usefulness of these methods. It also brings to light a number of new features of proanthocyanidin biochemistry which may have an important bearing on future studies.

RESULTS AND DISCUSSION

Earlier work [15] shows that proanthocyanidin polymers exist as chains of C(4)–C(8) [or C(6)] linked flavan-3-ol units, as would be predicted from the structures of procyanidin dimers [8–10]. Thus the monomer unit of the polymer chain may be based on either of two stereochemistries designated *cis* (**1**) or *trans* (**2**) and on either of two B-ring oxidation patterns, 3′,4′-dihydroxyphenyl (designated a procyanidin (PC) unit, **1a** or **2a**) or 3′,4′,5′-trihydroxyphenyl (designated a prodelphinidin (PD) unit, **1b** or **2b**). Therefore the polymer chains are based on four different structural units. Currently we can measure average stereochemistry and PC:PD ratios, but not subdivide the structure of the units further.

The structural data for 38 proanthocyanidin polymers are summarized in Table 1. All data are largely derived from the ^{13}C NMR spectra of polymers, except the terminal group composition which can only be determined by acid degradation [15, 16].

Solubility, and homogeneity of proanthocyanidin polymers

Virtually all the systematic knowledge of the phytochemistry and distribution of proanthocyanidins in the plant kingdom comes from the work of Bate-Smith [3, 4, 12, 17–20]. It is pertinent here to comment on the relationship of his methodology to that employed in our studies. Bate-Smith's procedure involves air-drying leaf tissue at 40°, finely milling it to

* There is now little doubt that the terms condensed tannin and proanthocyanidin polymer are interchangeable. However, it is probably preferable to reserve the former term to describe any hydrolysis-resistant, non-lignin, polyphenolic polymer capable of precipitating protein from aqueous solution.

Table 1. Phytochemical data on

Family	Plant	Organ
Filicales		
Cyatheaceae	*Cyathea dealbata* = *Alsophila tricolor*	frond (Summer)
		frond (Winter)
Dicksoniaceae	*Dicksonia squarossa*	frond
Coniferales		
Pinaceae	*Pinus radiata*	phloem
	Pinus radiata	middle bark
Dicotyledones		
Actinidaceae	*Actinidia chinensis*	leaf
Betulaceae	*Betula alba*	catkins
Ericaceae	*Vaccinium corymbosum*	unripe fruit
	Vaccinium corymbosum	ripe fruit
	Vaccinium oxycoccus	unripe fruit
Grossulariaceae	*Ribes grossularia*	unripe fruit
	Ribes nigrum	unripe fruit
	Ribes nigrum	leaf
	Ribes rubrum	leaf
	Ribes sanguineum	ripe fruit
	Ribes sanguineum	leaf
Hippocastanaceae	*Aesculus* × *carnea*	unripe fruit
	Aesculus hippocastanum	unripe fruit
Leguminosae	*Acacia pravissima*	leaf
	Lotus corniculatus	root
	Lotus pedunculatus	leaf
	Lotus pedunculatus	root
	Onobrychis viciifolia	leaf
Myrtaceae	*Feijoa sellowiana*	ripe fruit
Proteaceae	*Grevillea robusta*	leaf
	Grevillea rosmarinifolia	leaf
Rosaceae	*Chaenomeles sinensis*	unripe fruit
	Cotoneaster serotina	unripe fruit
	Cydonia oblonga	unripe fruit
	Fragaria annanasa cv 'Redgauntlet'	unripe achenes
	Fragaria annanasa cv 'Redgauntlet'	ripe achenes
	Malus pumila cv 'Granny Smith'	ripe fruit
	Photinia glabrescens cv *rubra* × *P. serrulata*	leaf
	Rosa centifolia cv	ripe hips
	Rosa centifolia cv	unripe hips
Salicaceae	*Salix fragilis*	leaf
	Salix caprea	catkins
Vitidaceae	*Vitis vinifera* cv 'Beaujolais'	unripe fruit
	Vitis vinifera × cv 'Siebel'	unripe fruit

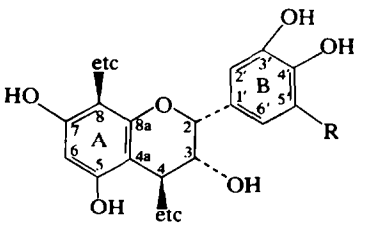
* Approximate estimate of MW.

e = (−)-epicatechin; c = (+)-catechin; egc = (−)-epigallocatechin; e-gallate = (−)-

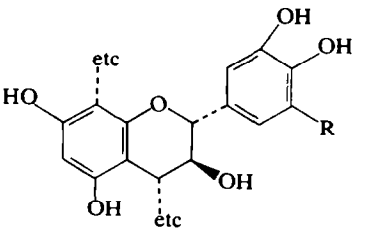
proanthocyanidin polymers

PC:PD	*cis*:*trans*	% Hydrolysable tannin	Terminal group	M_n
60:40	96:4	—	e:c:egc = 7:7:86	large
68:32	94:6			810
80:20	94:6	—	c = 100	3000*
52:48	74:26	—	c:egc = 60:40	2300
90:10	41:59	—	c:egc = 65:35	1750
88:12	97:3	—	e:c = 78:22	2100
84:16	82:18	—	e:c:egc = 20:70:10	1800
100:0	94:6	—	e:c = 30:70	3500
100:0	95:5	—	n.d.	n.d.
78:22	87:13	—	n.d.	1750
63:37	77:23	—	n.d.	2700
40:60	74:26	—	e:c:egc = 19:26:55	large
6:94	14:86	—	e:c:egc = 3:11:86	4300*
10:90	92:8	—	e:c = 60:40	3000*
23:77	72:28	—	c:egc = 9:81	2700
10:90	12:88	—	e:c:egc = 4:4:92	3300
100:0	93:7	—	e:c = 80:20	2200
100:0	97:3	—	e:c = 90:10	1750
45:55	73:27	36	n.d.	n.d.
80:20	87:13	—	n.d.	n.d.
20:80	80:20	—	c:egc = 30:70	large
23:77	73:27	—	e:c:egc = 10:67:22	6000*
23:77	87:13	—	e:c:egc = 22:38:40	3800
70:30	85:15	—	n.d.	n.d.
39:61	72:28	—	e:c:egc = 12:24:64	5000*
18:82	90:10	—	e:c:egc = 6:13:81	3300
100:0	94:6	—	e:c = 70:30	n.d.
93:7	90:10	—	n.d.	n.d.
100:0	95:5	—	e:c = 60:40	3000
100:0	76:24	37	n.d.	n.d.
100:0	74:26	26	n.d.	n.d.
100:0	93:7	—	n.d.	n.d.
100:0	97:3	—	c = 100	3800
90:10	76:24	33	c = 100	n.d.
90:10	72:28	37	n.d.	n.d.
95:5	87:13	—	e:c = 50:50	n.d.
78:22	38:62	—	n.d.	n.d.
87:13	92:8	—	e:c:egc = 40:39:21	3000*
85:15	95:5	—	e:c:egc:e-gallate 37:37:21:5	large

epicatechin 3-*O*-gallate; M_n = number-average MW. n.d. = Not determined.



1a R = H
1b R = OH



2a R = H
2b R = OH

100-mesh size and extracting it three times with boiling 50% aq. MeOH. The proanthocyanidins of the solution and residual leaf tissue are estimated by generating cyanidin or delphinidin chlorides and colorimetric determination [11]. The relative astringency in tannic acid equivalents is estimated by precipitation of blood protein ('haemanalysis') either in solution or in the residual plant material [11].

It is now evident that although the above procedures undoubtedly yield useful comparative data on relative tannin contents of plant tissue, they are rather questionable from a number of points of view. For instance, the solvent system used by Bate-Smith for proanthocyanidin extraction, 50% aq. MeOH, is a poor solvent for proanthocyanidin polymers. In fact, its lack of solvating power is the basis for purification of the polymers by adsorption chromatography on Sephadex LH-20 [16, 21]. In this procedure a crude polymer preparation is applied to a column of Sephadex LH-20 in 50% aq. MeOH and the proanthocyanidin is immediately adsorbed. Purification is achieved by subsequent washing with a large volume of the same solvent mixture which removes contaminating carbohydrate and lower MW phenolics. The purified polymer may then be eluted as a single narrow band with a small volume of Me_2CO–H_2O (usually 7:3 or 1:1). It is evident from this procedure that Me_2CO–H_2O mixtures are powerful solvents for proanthocyanidin polymer extraction and are even capable of breaking tannin-protein associations, a property that is common knowledge in the leather industry [22]. They are therefore the most suitable solvents for the extraction of the polymers from plant debris.

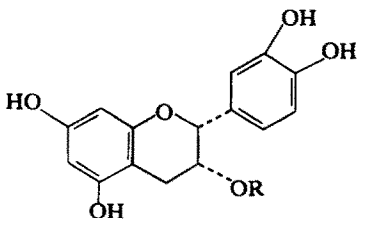
This may be convincingly demonstrated by TLC chromatography of the proanthocyanidins from *Aesculus hippocastanum* (a low MW polymer, see Table 1) and *Onobrychis viciifolia* (a high MW polymer [21]; having a value of M_n over twice as high, Table 1). The *Aesculus* polymer streaks to an R_f of *ca* 0.5 in water, whereas the *Onobrychis* tannin is virtually immobile. In MeOH–H_2O (1:1) both tannins streak considerably. All the *Aesculus* tannin moves off the origin, but approximately one-third of the *Onobrychis* polymer remains immobile. In contrast, both polymers are very mobile in Me_2CO–H_2O (either 1:1 or 7:3) and appear as very small, intense spots at the solvent front. This behaviour readily explains the observations of Bate-Smith, who has classified polymers according to their extractability [12, 17–20]. The proanthocyanidin from *Onobrychis* is classified as having a low extractability [17] in MeOH–H_2O whereas, in fact, it may be isolated in 95% yield by extraction with Me_2CO–H_2O [21]. In fact Bate-Smith's extractability index is merely a reflection of the poor solvating power of MeOH–H_2O mixtures. Extraction of polymers in boiling solvent is also questionable, in view of the known lability of proanthocyanidins in aqueous or alcoholic systems [8–10, 13].

Bate-Smith estimates total tannins by 'haemanalysis' [11]. This method is not specific for proanthocyanidin polymers, which may be conveniently estimated by the formation of the red vanillin complex in the presence of HCl. Broadhurst and Jones [23] have shown that the method is reproducible and free from serious background interference effects. We have corroborated [16] Broadhurst and Jones' [23] value of $E^{1\%}_{1\,cm}$ 280 for the complex with a proanthocyanidin polymer, using polymer preparations shown to be homogenous by ^{13}C NMR and C and H analysis. The value of $E^{1\%}_{1\,cm}$ 280 is applicable to freeze-dried polymer preparations, which contain approximately three molecules of water of hydration per monomer unit [16]. If tannin concentrations are to be estimated on a molar basis, the average MW for a monomer unit may be assumed to be 350 (to allow for the water of hydration). This method enables reliable estimates of proanthocyanidin homogeneity to be made on purified tannin preparations, or proanthocyanidin concentrations in solution.

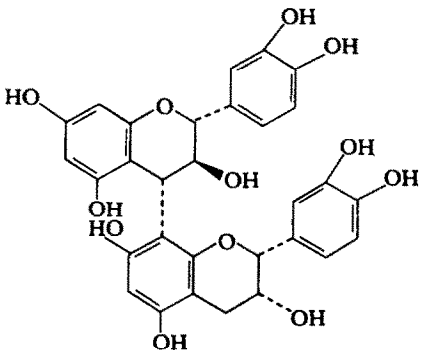
The vanillin estimation is most useful for those preparations which contain both hydrolysable tannins and proanthocyanidin polymers. These commonly co-occur, and five examples are noted in Table 1, the hydrolysable tannin being estimated by difference using the vanillin reaction. Hydrolysable tannins have identical solubility and Sephadex characteristics to proanthocyanidin polymers [16] and, so far, we have been unable to separate them. Hydrolysable tannins are readily detected in a purified tannin preparation by the presence of a carbonyl absorbance in the IR due to gallate or hexahydroxydiphenoyl ester moieties [16].

Hydrolysable tannins predominate in some of the species studied, particularly in the genus *Rubus*. Haslam and co-workers [24] have used blackberry (*Rubus fruticosus*) as a model system for biosynthetic studies using radioactively labelled cinnamate. However, the flavan-3-ols of blackberry are largely confined to (−)-epicatechin (**3**) and procyanidin B-4 (**4**) and no procyanidins were detected in the polymer fraction from fruit, stems, or leaves. This is consistent with the observation by Haslam [13] that blackberry fruit contain ellagitannins. Proanthocyanidins are completely absent from some *Rubus* varieties.*

* Plants surveyed include: *R. fruticosus* ('blackberry' or 'bramble'); *Rubus* × 'Aurora' (leaf); *Rubus* × (unnamed cultivated bramble leaf); *Rubus* × ('Boysenberry' leaf); *R. idaeus* ('raspberry', fruit).



3 R = H
5 R = gallate



4

Gallate esters

A complicating factor in the observation of a carbonyl in the IR of polymer preparations is the fact that it may not always be associated with the presence of significant concentrations of hydrolysable tannin. (−)-Epicatechin 3-*O*-gallate (**5**) is a constituent of *Camellia sinensis* leaves [25] and unripe grapes [26, 27]. Both the *Vitis* polymers (Table 1) displayed a small carbonyl absorption in the IR, but were judged to be homogeneous proanthocyanidin polymers by the vanillin reaction [16, 23]. The carbonyl is due to gallate ester functions attached to C-3 of the terminal (−)-epicatechin unit, and probably the *cis*-monomer units as well, as gallic acid is among the products of acid degradation of both *Vitis* polymers. Moreover, (−)-epicatechin 3-*O*-gallate (**5**) was among the thiolysis degradation products from the 'Siebel' polymer (Table 1).

Phytochemical implications of the results

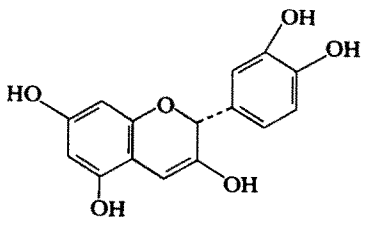
Oxidation pattern. The large number of polymers with a high proportion of PD units shown in Table 1 is not typical of proanthocyanidins. Our survey has deliberately included those plant tissues known to be rich sources of PD, because these polymers are of intrinsic interest due to their relative rarity and because data available on prodelphinidins as a whole is meagre [28]. There is little reason to doubt earlier surveys which have shown that PC polymers predominate in nature [1–4]. We have not so far isolated a polymer containing the rare propelargonidin unit.

Stereochemistry. The major feature of interest which emerges from the data in Table 1 is that the polymers normally appear to be based on units possessing predominantly the *cis* stereochemistry. Monomers with *trans* units predominate in only four polymers: *Pinus radiata* middle bark, *Ribes nigrum* and *R. sanguineum* leaf, and *Salix caprea* catkin. Even in these cases *cis* units are present in significant amounts. However, although *cis* units predominate, we have so far failed to isolate a polymer which contains *cis* units only [16].

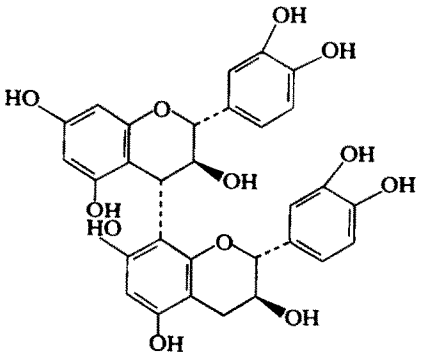
These observations may have considerable biological significance as they are fully consistent with the view proposed by Haslam and co-workers [13, 24] that the key intermediate in the biosynthesis of proanthocyanidins is a flav-3-en-3-ol (**6**) or its biological equivalent. This intermediate allows a plant to synthesize units of either *cis* or *trans* stereochemistry with equal facility through a common intermediate [13, 24]. The data in Table 1 are indirect, but powerful evidence for the existence of such an intermediate as the ability to synthesize either stereochemistry is probably present in all plant tissues so far studied.

The predominance of the *cis* stereochemistry is unexpected on the basis of present knowledge of the distribution of procyanidin dimers in plants [5–10]. Two of the polymers in this study were isolated from tissue where a dimer with a *trans*-PC unit (procyanidin B-3, **7**) was known to predominate: *Pinus radiata* phloem [29], and *Fragaria* × *anannasa* fruit [8–10]. Only further surveys will reveal whether or not this is a general feature of proanthocyanidin biochemistry.

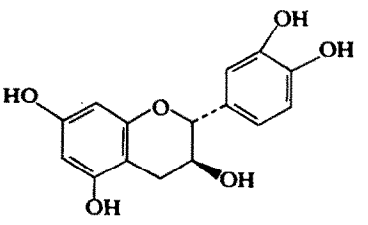
Terminal units. As was concluded by Haslam [13] for procyanidin dimers it is common to find (+)-catechin (**8**) or (−)-epicatechin (**5**) linked to a PC unit with the opposite stereochemistry. This is evidently



6



7



8

equally true for prodelphinidin dimers [28]. Such a situation is common for proanthocyanidin polymers too. In fact it is relatively rare to observe a unique flavan-3-ol as a chain-terminating unit. Examples of polymers possessing a terminal unit predominately of the opposite stereochemistry to the monomers are: *Dicksonia*, *Vaccinium corymbosum*, *Ribes nigra* and *R. sanguineum* leaf, *Lotus pedunculatus* root, *Rosa* and *Photinia*.

Number average molecular weights, M_n. The values of M_n of polymers isolated in this survey cover a very wide range (see Table 1), from chains containing, on average, only a few units, such as *Cyathea dealbata* (fronds, winter), to chains whose average length is too high to be assessed reliably by ^{13}C NMR. The upper limit of M_n which may be observed by ^{13}C NMR is dependent on a number of factors [16] and it is not solely dependent on the magnitude of M_n. However, some of the polymers undoubtedly possess values of M_n of up to several thousand. The general range of M_n encountered is therefore consistent with the long held view that condensed tannins are water-soluble polyphenols whose MW is in the range 500–3000 [30].

Recent work [16] also shows that the polymers, once purified and freed from other organic contaminants, are always readily and completely soluble in water, regardless of MW. Solubility problems associated with condensed tannins are probably due to the presence of small amounts of protein or carbohydrate, which are both capable of binding to these polymers in aqueous solution, as discussed earlier.

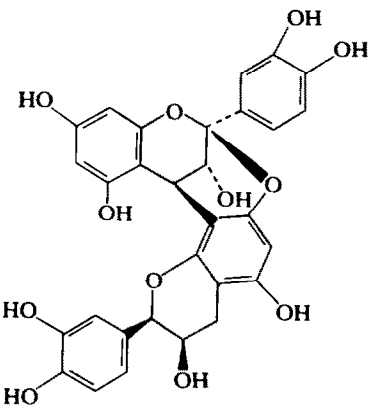
A compilation of the MW, measured by ultracentrifuge, of a range of purified polymers isolated from herbaceous legumes has been published [21]. At first sight these data appear to imply that the polymers have a much higher MW than the M_n values determined by ^{13}C NMR. However, the ultracentrifuge yields z-average MW (M_z) and the relationship between M_n and M_z is not straightforward, being strongly dependent on the shape of the MW distribution. Moreover, proanthocyanidin polymers undoubtedly self-associate in aqueous solution [16] further complicating the interpretation of these data.

Biological and systematic implications

It is evident, even from this survey, that the structures of proanthocyanidin polymers are very much the same, regardless of the type of plant tissue, or the evolutionary status of a plant. It is therefore not likely that more extensive surveys of polymer structures will be of much value to plant systematics in the broad sense.

However, detailed knowledge of polymer structures will be of undoubted taxonomic value within narrower, such as family, groups. This is well illustrated by the very limited survey made of the genus *Ribes*. All polymers in the narrow group studied contain a high proportion of PD units, which, as Bate-Smith [18] has shown, is typical of this genus.

The structures of the polymers from the leaves of *R. nigrum* and *R. sanguineum* are virtually identical, whereas that from *R. rubrum* contrasts in containing largely *cis* units. This is consistent with *R. rubrum* being in a separate section (*Ribesia*) from the other species, and may also imply that *R. nigrum* and *R. sanguineum* (which are in *Eucoreosma* and *Calobotyra*, respectively) are more closely related than previously suspected, especially as their fruit polymers also possess a very similar structure. An intriguing feature of this pair of species is that the polymer in the leaves has predominantly a *trans* stereochemistry, the opposite to that in the fruit. This observation is totally unexpected and implies that polymer synthesis must operate under quite different controls in the fruit and leaves. The PC content of *R. grossularia* fruit polymer is much higher than in the other *Ribes* fruits studied. This is consistent with it being in a separate section from the other species (*Eugrossularia*).



9

The proanthocyanidins from *A.* × *carnea* and *A. hippocastanum* fruits both include the unusual doubly-linked dimer proanthocyanidin A-2 (**9**), and trimers of a similar structure [24, 31]. Proanthocyanidin A-2 has been used as a taxonomic marker by Bate-Smith [12] who has commented on its scattered distribution in plants.

It could be expected that A-type linkages would also occur in the polymers isolated from *Aesculus*. However, the polymer isolated from either species is in fact a normal B-type linked polymer, similar to many other predominantly PC and *cis* polymers isolated from other sources. As shown in Fig. 1 the ^{13}C NMR spectrum of the *A. hippocastanum* polymer is identical to that of a similar polymer from *Actinidia chinensis*, apart from the obvious difference in M_n manifested by the small signal for the terminal group C-3 resonance in the latter polymer's spectrum. No products consistent with the presence of proanthocyanidin A-2 (**9**) type structures were detected on acid degradation [25] or by degradation with phloroglucinol or phenylmethanethiol in mildly acid conditions. These results suggest that proanthocyanidin A-2 (**9**) is a metabolic curiosity and its formation is subsidiary to the main biosynthetic pathway forming the polymers.

There have been many qualitative observations made on the fate of tannins during the aging or maturing of plant tissue, especially with respect to fruit ripening [13]. It has been observed, quite commonly, that the concentration of proanthocyanidin polymers drops as a fruit matures [13]. However it can often be argued that the concentration remains fairly constant on a per fruit basis [13, 32] and sometimes an increase

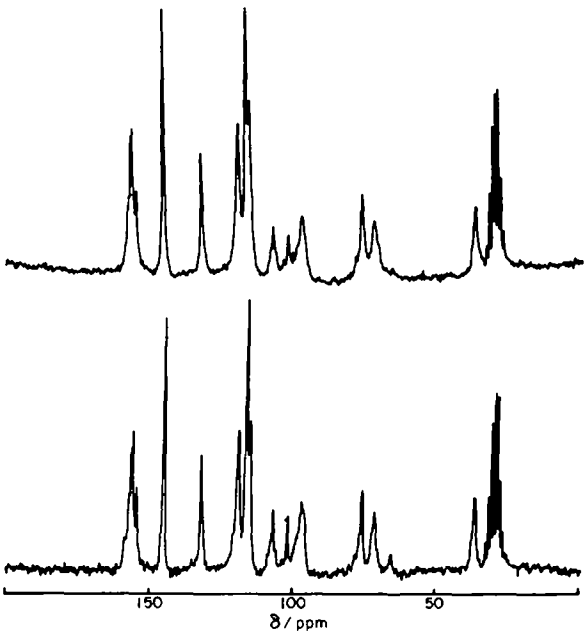


Fig. 1. ^{13}C NMR of proanthocyanidin polymers in Me_2CO-d_6-H_2O. Upper spectrum: *Actinidia chinensis*. Lower spectrum: *Aesculus hippocastanum*.

is observed near the end of ripening [32, 33]. Obviously the topic is open to further study. It has also been suggested by Goldstein and Swain [34], that the MW of tannins increase as fruit or leaves mature, and that their solubility and hence astringency decreases.

There are some observations pertinent to the above arguments that have been made in this survey. Although limited, they are of considerable interest as they are based on a precise knowledge of the structural changes that have occurred in the polymers during tissue aging. Examples are as follows: the polymers from the fronds of the tree fern *Cyathea dealbata* were isolated in mid-summer, when the fronds were rapidly growing, and compared with the polymer present in fronds in mid-winter when growth was essentially dormant. The polymer structures were virtually identical except for an enormous difference in M_n, the value being very high in mid-summer, but dropping in mid-winter to the lowest value recorded for a polymer in this study, 810, equivalent to a mean chain length of *ca* 3 units.

The polymers from ripe and unripe fruits of blueberry and strawberry have been isolated (Table 1). The ^{13}C NMR spectra of the polymers and other properties were in each case virtually identical. The strawberry polymers also contained similar proportions of hydrolysable tannin. These results imply that there is no significant change in proanthocyanidin polymer synthesis in fruit, on ripening, apart from a lowering of concentration on a weight basis. The yield of purified tannin isolated from ripe strawberries and blueberries was 65 and 27% respectively of that isolated from unripe fruit.

The above observations on the polymers from fern fronds, and also those from fruit, are in direct conflict with the view expressed by Goldstein and Swain [34]. It may ultimately be proved that the MW of the polymers actually decreases in senescent leaves. Indeed, whereas the polymers are present in the stems and leaves of some deciduous trees, such as *Salix*, in summer, often only cyanidin and monomeric catechins are present in the stems after leaf drop.

Interesting examples of structural differences in polymers from different tissues of the same plant are those already discussed for *Ribes* leaves and fruit, and those observed for *Pinus radiata* phloem and bark. The latter example is particularly interesting, as the middle bark is derived from the phloem by formation of suberized tissue and consequent cell desiccation [35], and therefore the bark tannin must ultimately be derived from the phloem. This change in polymer structure is therefore somewhat unexpected and may imply that the phloem contains a mixed polymer, one containing largely PC-*trans* units and the other largely PD-*cis* units. The PD polymer might then be selectively degraded via its labile trihydroxylated B-ring by either enzymic or aerial oxidation, or both, following cell desiccation. The net result would be that the polymer actually found in the bark is an oxidative degradation production formed from the original phloem polymers.

EXPERIMENTAL

The proanthocyanidin polymers were isolated from fresh plant material with aq. Me_2CO and purified by adsorption chromatography on Sephadex LH-20 [16, 21]. The ^{13}C NMR measurements were performed on a Varian FT-80A instrument in D_2O–H_2O or Me_2CO-d_6–H_2O. The vanillin–HCl colorimetric estimation of proanthocyanidins was carried out as described in ref. [23].

Acknowledgements—To Dr. R. H. Newman for ^{13}C NMR spectra; Mr. L. A. Porter for the supply of berry fruit and Dr. W. T. Jones for helpful discussions.

REFERENCES

1. Robinson, G. M and Robinson, R. (1933) *Biochem. J.* **27,** 206.
2. Robinson, G. M. and Robinson, R. (1935) *J. Chem. Soc.* 744.
3. Bate-Smith, E. C. (1954) *Biochem. J.* **58,** 122.
4. Bate-Smith, E. C. and Lerner, N. H. (1954) *Biochem. J.* **58,** 126.
5. Weinges, K., Kaltenhauser, W., Marx, H.-D., Nader, E., Nader, F., Perner, J. and Seiler, D. (1968) *Annalen* **711,** 184.
6. Weinges, K., Bahr, W., Ebert, W., Goritz, K. and Marx, H.-D. (1969) *Fortschr. Chem. Org. Naturst.* **27,** 158.
7. Weinges, K., Baehr, W., Theobold, H., Wiesenhuetter, A., Wild, R. and Kloss, P. (1969) *Arzneim.-Forsch.* **19,** 328.
8. Thompson, R. S., Jacques, D., Haslam, E. and Tanner, R. J. N. (1972) *J. Chem. Soc. Perkin Trans.* 1, 1387.
9. Fletcher, A. C., Porter, L. J., Haslam, E. and Gupta, R. K. (1977) *J. Chem. Soc. Perkin Trans.* 1, 1628.
10. Gupta, R. K. and Haslam, E. (1978) *J. Chem. Soc. Perkin Trans.* 1, 892.
11. Bate-Smith, E. C. (1973) *Phytochemistry* **12,** 907.
12. Bate-Smith, E. C. (1975) *Phytochemistry* **14,** 1107.
13. Haslam, E. (1977) *Phytochemistry* **16,** 1625.
14. Haslam, E. (1974) *Biochem. J.* **139,** 285.

15. Czochanska, Z., Foo, L. Y., Newman, R. H., Porter, L. J. Thomas, W. A. and Jones, W. T. (1979) *J. Chem. Soc. Chem. Commun.* 375.
16. Czochanska, Z., Foo, Lai Yeap, Newman, R. H. and Porter, L. J. (1980) *J. Chem. Soc. Perkin Trans.* 1, (in press).
17. Bate-Smith, E. C. (1973) *Phytochemistry* **12,** 1809.
18. Bate-Smith, E. C. (1976) *Biochem. Syst. Ecol.* **4,** 13.
19. Bate-Smith, E. C. (1977) *Phytochemistry* **16,** 1421.
20. Bate-Smith, E. C. (1978) *Phytochemistry* **17,** 267.
21. Jones, W. T., Broadhurst, R. B. and Lyttleton, J. W. (1976) *Phytochemistry* **15,** 1407.
22. Russell, A. E., Shuttleworth, S. G. and Williams-Wynn, D. A. (1968) *J. Soc. Leather Trades Chem.* **52,** 459.
23. Broadhurst, R. B. and Jones, W. T. (1978) *J. Sci. Food Agric.* **29,** 788.
24. Jacques, D., Opie, C. T., Porter, L. J. and Haslam, E. (1977) *J. Chem. Soc. Perkin Trans.* 1, 1637.
25. Forrest, G. I. and Bendall, D. S. (1969) *Biochem. J.* **113,** 741.
26. Weinges, K. and Piretti, M. V. (1971) *Annalen* **748,** 218.
27. Czochanska, Z., Foo, Lai Yeap and Porter, L. J. (1979) *Phytochemistry* **18,** 1819.
28. Foo, Lai Yeap and Porter, L. J. (1978) *J. Chem. Soc. Perkin Trans.* 1, 1186.
29. Porter, L. J. (1974) *N.Z.J. Sci.* **17,** 213.
30. Haslam, E. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds) p. 528. Chapman & Hall, London.
31. Jacques, D., Haslam, E., Bedford, G. R. and Great-banks, D. (1974) *J. Chem. Soc. Perkin Trans.* 1, 2663.
32. Singleton, V. L. and Esau, P. (1969) in *Phenolic Substances in Grapes and Wine, and Their Significance*, pp. 118–119. Academic Press, New York.
33. Somers, T. C. (1976) *Vitis* **14,** 269.
34. Goldstein, J. L. and Swain, T. (1965) *Phytochemistry* **4,** 185.
35. Jensen, W., Fremer, K. E., Sierila, P. and Wartiovaara, V. (1963) in *The Chemistry of Wood* (Browning, B. L., ed.)